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OBSERVATION OF CHANGES IN THE ACOUSTIC IMPEDANCE OF THE EAR AS AN AID TO THE DIAGNOSIS OF PARALYSIS OF THE STAPEDIUS MUSCLE

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The aim of this in estigati n was to lucidate whether lifting the upper eyelids in patients with paralysis of the stapedius muscle resulted in impedance changes that were so characteristic that they could be used in the topical diagnosi of peripheral facial paralysis. Twenty-three patients with unilateral peripheral facial paralysis were investigated. Tactile and acoustic stimulation and lifting the upper eyelids elicited n rmal changes in impedance bilaterally in 11 cases, indicating lesion of the facial nerve peripheral to the stapedial nerve In the remai i g 12 patients, paralysis f the stapedius muscle demonstrated a lesion of the facial nerve central to the stapedial nerve This condition is characterized by

1 No changes in impedance to tactile and acoustic stimulation.
2. Abnormally large changes in impeda ce to lifting the upper eyelids (voltmeter needle deflection > 100)

The facial erve palsy was transitory in all cases and the pathological *impedance changes gradually became normal upon recovery f the* facial nerve fun ti ni g.

Peripheral paralysis of the facial nerve is a common condition. Location of the lesion is of great importance for etiological diagnosis and treatment. The stapedial nerve is the first motor branch to leave the main nerve stem information of the stapedius muscle function will therefore be valuable in the topical diagnosis of the facial nerve lesion (Jepsen, 1955 Klockhoff 1961 Feldman, 1964 and others)

The function of the stapedius muscle in man is usually tested by observation of changes in the acoustic impedance of the ear caused by reflex con traction of the stapedius muscle This is normally elicited by acoustic stim ulation of the contralateral ear Jepsen (1955) considered no change in the impedance on the paralysed side after acoustic stimulation to be a sign of a suprastapedial lesion of the f cial nerve with paralysis of the stapedius muscle. However as menti ned by Metz (1946) Jepsen (1955) Terkildsen & Scott Nielsen (1960) Klockhoff (1961) Feldman (1964) and others, this finding could also be due to the following

1 Cochlear stimulation at insufficient intensity levels on the opposite side (e.g. hearing loss of conductive type)

2 Damage to the afferent part of the reflex arch (e.g hearing loss of retrocochlear type)
3 Disturbance of the middle ear function on the paralysed side (low pressure in the middle ear fixation of the stapes, incus or malleus discontinuity of the ossicular chain, etc.)

A hearing loss of conductive or retrocochlear type can generally be diag nosed by audiometric tests It is considerably more difficult to determine whether a lack of impedance change to acoustic stimulation is due to paralysis of the stapedius muscle or to a disturbance of the middle ear function

Klockhoff (1961) has investigated the function of the middle ear in patients with peripheral facial paralysis exhibiting no change in impedance on acoustic stimulation by using "the tensor reflex test (Reflex activity in the tensor tympani muscle elicited by directing jets of air towards the ocular region.) He concludes "If stimulation with the orbital air jet also fails to produce a response, the conclusion will be that the absence of a stapedius response affords no guidance in localization of the facial nerve lesion If on the other hand, a tensor response occurs and is of the type usually found in normal middle ears, it will be possible to infer with greater assurance that a stapedius reflex has been abolished by a suprastapedial facial nerve lesion."

In normal ears, lifting the upper eyelids produces a defensive reaction leading to changes in the acoustic impedance These impedance changes are elicited by simultaneous contraction of both the stapedius and the tensor tympani muscles (Djupesland, 1967) Since these muscles are antagonists in the anatomical sense (Jepsen 1955) it is likely that lifting of the upper eyelids will lead to different impedance changes in normal ears versus ears with a paralysed stapedius muscle. The object of this investigation was to test this hypothesis.

MATERIAL AND METHODS

The investigation included 23 patients (13 female and 10 male) 12–65 years of age. All exhibited unilateral peripheral facial paralysis. The clinical diagnosis is shown in Table 1

The facial paralysis was transitory in all cases. The ear drum was nor mal in 20 cases. In three patients with zoster oticus the tympanic membrane on the affected side was slightly swollen and reddend during the acute stage, but returning to normal later The mobility of the tympanic membrane appeared normal

Pure tone audiometry indicated normal hearing in 18 cases. Five patients exhibited a moderate high frequency "dip" as a result of acoustic trauma in both ears.

The middle ear pressure was measured as described by Terkildsen & Thomsen (1959) and found to be normal in all cases.

Changes in the acoustic impedance of the ear were recorded using an

Table 1

Clinical diagnosis	Number of patients
"Bell palsy"	14
Zoster oticus	3
Traumatic facial paralysis	5
Parotitis	1
Total	23

electroacoustic impedance indicator This apparatus is a modification of the electroacoustic impedance measuring bridge described by Terkildsen & Scott Nielsen (1960) its construction and the mode of action have been reported previously (Djupesland, 1964 1967)

During the observation period of 18 months repeated observations of impedance changes were made on both ears in all the patients. The impedance indicator was set at maximum sensitivity ("sensitivity setting 10") after counterbalancing, the following tests were carried out

(a) Touching of the skin around the opening of the homolateral external auditory meatus with a thick twist of cotton wool

(b) Acoustic stimulation of the contralateral ear with Bárány s noise box. The noise box was held near the opening of the external auditory meatus but not so near that the skin was touched.

(c) Lifting of the upper eyelids. The lifting of the eyelids was carried out in such a way that a defensive reaction, including visible contraction of various muscles of the head and neck, was elicited.

RESULTS

Observations made at the first examination of all patients are shown (Table 2)

Table 2. *Observation of impedance changes during first examination*

Number of patients in parentheses

	Impedance indicator placed on the paralysed side		Impedance indicator placed on the non-paralysed side
Acoustic stimulation to the contralateral ear	+ (11)	(12)	+ (23)
Tactile stimulation to the homolateral ear	+ (11)	− (12)	+ (23)
Lifting the upper eyelids	(11)	+ (12)	+ (23)

The impedance indicator has a voltmeter scale graded from 0–100. Needle deflection on this meter reflects the degree of imbalance caused by changes in impedance which were observed and recorded according to the following key

− = Absent (no deflection of the voltmeter needle)

\+ Small (voltmeter needle deflection < 30)

\+ + Normal (voltmeter needle deflection 35–95)

\+ + + = Abnormally large (voltmeter needle deflection > 100)

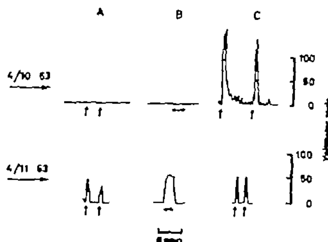


Fig. 1. Changes in the acoustic impedance of the ear recorded in the acute stage of transitory peripheral facial paresis (4.10.63) and after the paresis had disappeared (4.11.63). (A) Touching the skin around the homolateral external auditory meatus with a twist of cotton wool (arrows indicate moment of stimulation); (B) acoustic stimulation of the contralateral ear with Bárány's noise box (arrow indicates time and duration of stimulus); (C) lifting the upper eyelid (arrows indicate moment of stimulation).

DISCUSSION

In 11 of the patients a normal stapedius muscle function was found in both ears indicating that the lesion of the facial nerve was peripheral to the stapedial nerve.

In the remaining 12 patients paralysis of the stapedius muscle was demonstrated indicating that the lesion of the facial nerve was central to the stapedial nerve. This paralysis was diagnosed on the basis of the following findings:

1. No change of impedance to tactile and acoustic stimulation.
2. Abnormally large changes in impedance to lifting the upper eyelids.

The changes in impedance brought about by lifting the upper eyelids were in most cases more than twice as large on the paralysed side as those recorded on the opposite side (Fig. 1).

The elicited contraction of the tensor tympani muscle, which in these cases is not counteracted by the paralysed stapedius muscle, leads to the abnormally large changes in impedance.

The stapedius muscle seemed to regain its function over a varying length of time. This was manifested by a return of the stapedius muscle reflex elicited by acoustic and tactile stimulation. At the same time it was observed that the impedance changes elicited by lifting the upper eyelids diminished in size. It was not until the mimetic musculature had regained its normal function that lifting the upper eyelids resulted in similar changes of the impedance as in normal ears (Fig. 1).

CONCLUSION

Paralysis of the stapedius muscle with an otherwise normal middle ear function is characterized by

(*a*) No change in the acoustic impedance of the ear to tactile and acoustic stimulation.

(*b*) Abnormally large changes in the acoustic impedance to lifting the upper eyelids (voltmeter needle deflection ≥ 100)

ZUSAMMENFASSUNG

Die Absicht dieser U tersuchung war hervorzubri gen, ob das Heben von den oberen Augenlidern bei Patienten mit Stapediusmuskellähmung Impedanz Änderungen verursachen die so typisch seien, dass sie in der topischen Diagnose der peripheren Facialislähmung verwendet werden können. Dreiundzwanzig Patienten mit unilateraler Faci lislähmung sind untersucht worden. In 11 Fällen sind normale Änderungen in der akustischen Impedanz beider Ohren durch taktile und akustische Reizung und beim Heben der oberen Augenlider ausgelöst worden, und diese Befunde beweisen eine Verletzung des Nervus Facialis peripher vom Stapediusnerven. In den übrigen 12 Patienten ist eine Verletzung des Nervus Facialis zentral vom Nervus Stapedius durch Lähmung des Musculus zu erklären, erkennbar durch

1 K in Impedan Änderungen bei taktile und akustischer Reizung.

2. Abnormal grosse Impedan Änderu gen beim Heben der oberen Augenlider

Die F cialislähmung war in allen Fällen vorübergehend, und die pathologischen Impeda Änderungen sind allmählich wieder normal geworden, als die Facialislähmung zurückging.

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EFFECTS OF HIGH INTENSITY IMPULSE NOISE AND RAPID CHANGES IN PRESSURE UPON STAPEDECTOMIZED MONKEYS

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To determine the effects of impulse noise and rapid changes in pressure upon stapedectomized patients, 40 monkeys were subjected to the stapedectomy procedure and later exposed to gunfire or rapid changes in pressure in an altitude chamber. Two different prostheses were used, half the monkeys receiving the polyethylene strut and vein graft, the other half getting a stainless steel piston prosthesis. Immediate post-exposure examination of the monkeys was made by reflecting the drums. No experimental disarticulation of the prostheses was observed, nor were there any behavioral manifestations of vestibular pathology. No significant differences were observed between the two different prostheses used. On the basis of this experiment, no valid reason for drastic duty limitation of stapedectomized patients can be seen.

Today we find the stapedectomy procedure the treatment of choice in the management of otosclerosis. The widespread incidence of otosclerosis, plus the popularity of the stapedectomy procedure, have resulted in a great number of persons undergoing this operation. The relative newness of the procedures and the difficulties attendant upon research in this area in part account for the dearth of research on the subsequent effects of this procedure upon the hearing abilities and susceptibilities of those so treated. Investigations have been made of the susceptibility of stapedectomized patients to noise induced temporary threshold shifts (Steffen & Nixon, 1963; Fletcher & King, 1963; McGee *et al.* 1962). So far, the findings appear to indicate no heightened susceptibility to acoustic insult. However, at best many problems beset the researcher in this endeavor. One critical problem is that of securing a proper control group against which to compare a stapedectomized population. Ideally, the control group should differ from the experimental group only in that they have not been stapedectomized. Certainly, auditory acuity of the two groups should be the same, because auditory acuity is a limiting factor in noise-induced temporary threshold shifts (Glorig *et al.* 1961). This requirement is exceedingly difficult to meet, and as a result, extreme caution must be observed in evaluating the results of studies where this condition was not met.

Several problems should be considered in evaluating the hazards of the

environment to the stapedectomized person. Impulse noise, with its precipitous rise time and typically high overpressure would logically be thought to pose more of a problem than would continuous noise, not only because of the possibility of noise induced hearing loss, but also because of the possibility of the inertia of the prosthesis-ossicular chain interacting with the steep rise time of the impulse to result in disarticulation of the prosthesis, or more drastic damage Andersen *et al* (1964) measured the transmission of sound before and after insertion of stapedial prostheses in cadaver temporal bones. They found no prosthesis weight-frequency related effects. They did, however note that exposure to "violent" sounds resulted in disarticulation of the prostheses. They also observed that at such high levels (in excess of 100 dB) the prosthesis did not follow the movement of the incus. Subjective reports are frequently encountered suggesting that changes in altitude, such as those found in unpressurized or suddenly de-pressurized airplane cabins, result in failure of the prostheses. However one might think that if the patient were able to equalize the pressure Le "clear" his ears, danger from this source would be minimized.

The Armed Forces are a particularly lucrative source of both problems and research possibilities with regard to stapedectomies. Gunfire noise exposure is, of course, typical of military personnel and with the increasing use of aircraft of all kinds, and of airborne operations involving parachuting, a large number of persons are exposed to rapid changes in pressure Problems of this nature suggested the research to be reported in the succeeding sections. Specifically an investigation was made of the effects of impulse noise and sudden changes in pressure (simulating altitude changes) upon stapedectomized monkeys. Monkeys were used because of both the ethical and practical considerations involved. Also, the ear of the monkey appears to be quite similar to that of man and it was believed that it would respond in much the same way as the human ear

METHOD

A total of 40 Cebus monkeys were procured and subjected to surgery The Cebus monkey a New World species, was used because its middle ear is quite accessible to surgery unlike that of the Old World monkeys, and is markedly similar to that of man Both ears of each animal were operated with the same type of prosthesis placed in each ear Half the animals were treated with a modified Shea (1960) technique utilizing a No 90 polyethylene strut and vein graft (PE+V) while the other half had a stainless steel piston (SSP) made especially to fit the Cebus monkey This prosthesis was identical to those used in humans except for its size being considerably smaller Five of the surgically completed animals expired subsequent to surgery from non surgical causes so th t our experiment was performed on a population of 35 animals. These animals were randomly divided into three groups. Group I was exposed to high intensity impulse noise on two occa

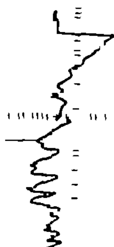


Fig 1 Tra l g f oscillogram of impulse produced by M 14 rifl Each horizontal divi sion = 100 μsec peak = 160 dB.

sions, to machine gun fire and to 00 mm cannon fire See Fig 1 for an oscilloscope tracing of the impulse from an M 14 rifle. The machine gun pulse will be about the same as that of the M 14 while cannon fire will have a slightly slower rise time and somewhat longer duration Peak pressure for the M 14 approximates 160 dB, with duration of the positive phase of about 2.2 msec. Group II was exposed to rapid changes in pressure simulat ing changes in altitude while Group III was the control group They were operated, kept with and treated like the experimental groups except that they were not experimentally exposed to either of the variables.

Group I, which consisted of 12 animals (24 ears) was exposed to 2000 rounds of fire from an M 73 7 62 cal machine gun. The fire was in 5 second bursts with a 5 second interval between bursts. All animals exposed had had at least two months to recover from surgery Firing was done on an open range in the field The quasi peak sound pressure level (SPL) measured inside the animal s retention cage, averaged 164 dB Sound measurement was accomplished using a General Radio Model 1551 B sound level meter a Massa 141 B microphone and a General Radio Model 1556-B im pact noise analyzer Variability of the pressure was ± 2 dB All animals were tranquilized two hours prior to exposure injection of 1 mg/kg body weight of Serylan (phencyclidine hydrochloride) in order to minimize activity during exposure (such as fighting, jumping, and other activities that might produce deleterious effects)

Group II also 12 animals, 24 ears, was placed in an altitude chamber and ascended to a pressure equivalent altitude of 30 000 feet then descended, free-fall, to 1200 feet. This was repeated three times with 2 minute intervals between runs. It would appear that Eustachian tube function in monkey and in man is the same Therefore no sedation or tranquilization was given this

Table 1 *Group I—Gunfire*

TM = Tympanic membrane MM = Middle ear mucosa. OW = Oval window
Tympanic membrane. Normal (N) = Self-explanatory Slight injury = Hyperemia in vascular strip area. General injury = Entire tympanic membrane hyperemic.
Original results. Self-explanatory
Middle ear mucosa. No reaction (NR) = Self-explanatory Slight reaction = Generalized injection. Moderate reaction = Some petechiae Frank hemorrhage = Self-explanatory
Oval window: Slight = Very little reaction in the drum or the vein around the prosthesis. Moderate = Somewhat greater reaction around prosthesis. Marked = Drum plastered to incus with much reaction around prosthesis.

Left ear: Post exposure appearance of the TM	Original results	Reaction of the MM	Reaction of the OW due to surgery	Right ear: Post exposure appearance of the TM	Original results	Reaction of the MM	Reaction of the OW due to surgery
Polyethylene strut and vein graft							
N	Originally slipped tube	NR	Slight	N	Good	NR	Slight
N	Good	NR	Moderat	N	Good	NR	Slight
N	Good	NR	Marked	N	Good	NR	Slight
N	Good	NR	Slight	N	Good	Moderate	Moderat
N	Good	NR	Slight	N	Good	NR	Slight
N	Good	NR	Slight	N	Good	NR	Slight
Stainless steel piston							
N	Good	NR	Slight	N	Good	NR	Slight
N	Good	NR	Slight	General injury	Good	Moderat Question in middle ear	Moderat of perilymph
N	Good (poor crimp, no slipping)	NR	Slight	N	Good	NR	Moderat
N	Good	NR	Slight	N	Good	NR	Slight
N	Good	NR	Slight	N	Good	NR	Slight
N	Good	NR	Moderat	N	Good	NR	Slight

group as we were afraid such treatment might interfere with the animals ability to equalize pressure

Group III 11 animals and 22 ears, was housed with the other animals, treated just like them, but not exposed to gunfire or pressure change

Immediately after exposure, the animals in Groups I and II were anesthetized and had their tympanic membranes reflected in order to determine the effects of the exposure upon the tympanic membrane, ossicular chain prosthesis, middle ear mucosa, and the oval window reaction. At a later date the animals of the control group, Group III were similarly scrutinized.

Table 2 *Group II—Altitude*

No perforations seen in any ears. For xplanation, see Table 1

Left ear: Post exposure appearance of the TM	Left ear: Original results	Left ear: Reaction of the MM	Left ear: Reaction of the OW due to surgery	Right ear: Post exposure appearance of the TM	Right ear: Original results	Right ear: Reaction of the MM	Right ear: Reaction of the OW due to surgery
Polyethylene strut and vein graft							
General I Jury	Good	Frank hemorrhage	Slight	Slight	Good	Slight	Slight
N	Good	NR	Slight	Slight	Good	Slight	Slight
N	Good	NR	Slight	Slight	Good	Frank hemorrhage	Slight
Slight	Good	Moderate	Slight	Slight	Good	NR	Slight
N	Good	NR	Slight	N	Slipped	NR	Slight
Slight	Slipped	Slight	Slight	Slight	Good	Frank hemorrhage	Slight
Stainless steel piston							
Slight	Good	Moderat	Slight	Slight	Good	Moderat	Moderat
Slight	Good	Moderate	Moderate	Slight	Good	NR	Moderate
Slight	Good	Frank hemorrhage	Moderat	N	Poor crimp	NR	Slight
General I Jury	Good	Moderat	Moderat	Slight	Good	Moderate	Marked
Slight	Good	Moderat	Slight	N	Good	Slight	Slight
N	Good	Slight	Slight	N	Good	NR	Slight

RESULTS

The overall results were surprisingly good from a clinical point of view i.e., the prostheses were extremely resistant to disarticulation so much so that none were experimentally interrupted. The observed reactions of the tympanic membrane, middle ear mucosa, and oval window were also remarkably mild. Both the PE+V and the SSP did well The data do not seem to differentiate between the two as far as we can tell. Complete results of the experiment are presented in Tables 1 2, and 3 As can be seen, gunfire had no observable effect upon the prostheses and little observable effect upon the tympanic membrane, middle ear mucosa, and the oval window Also, no behavioral effects of vestibular trauma were seen Obviously in view of the severity of the impulse noise to which these animals were exposed, more than a human would normally ever receive we need not fear failure of the prostheses from this source However it is equally plain that we cannot on

Table 3 *Group III—Controls*[a]

For explanation, see Table 1

Left ear				Right ear			
Post exposure appearance of the TM	Original results	Reaction of the MM	Reaction of the OW due to surgery	Post exposure appearance of the TM	Original results	Reaction of the MM	Reaction of the OW due to surgery
Polyethylene strut and vein graft							
N	OK	OK	OK	N	OK	OK	OK
N	OK	OK	OK	N	Slipped immediately post-op.	OK	OK
N	OK	Moderat	OK	N	OK	Moderat	OK
N	OK	OK	OK	N	Poor operative connection	OK	OK
N	OK	OK	Moderat	N	OK	OK	OK
N	OK	OK	Moderat	N	OK	OK	OK
N	OK	OK	OK	N	OK	OK	Moderat
Stainless steel piston							
N	Good	NR	N	N	Riding high, poor crimp	NR	N
N	OK			N	OK	OK	OK
N	OK	OK	OK	N	OK	OK	OK
N	OK	OK	OK	N	OK	OK	OK

[a] Some surgically completed animals expired from non-surgical causes, so control group slighted to allow correct experimental groups.

the basis of this experiment, say anything about the damage to the *hearing* of the animal, only that the prostheses did not fail. In a further effort to promote failure of the prostheses by impulse noise six animals of Group I were exposed to 12 rounds of gunfire from a 90 mm cannon. The average quasi-peak SPL, measured inside the cage, was in excess of 190 dB. Again, we failed to produce drastic results. The one response noted was a marked reaction of the middle ear mucosa in one animal. No other negative results were observed.

The effects of altitude (pressure) as seen in Table 2, were somewhat more noticeable. For example, Table 1 shows that in Group I, the appearance of the tympanic membrane was normal for all subjects. In Group II, however, only eight of the membranes appeared normal; the rest varied from a slight to a generally injected appearance. Similarly, the middle ear mucosa was normal in Group I, while in Group II, only seven were normal, with the remaining 17 varying from a slight reaction to frank hemorrhage. The oval window reaction observed in the two groups was about the same. As we

examine these data we are *inescapably drawn to the conclusion that* while neither of these experimental variables really wreaked havoc with the prostheses, the effects of the *pressure change were considerably more marked.* It is significant we believe, that no failures of the prostheses were induced. The procedures are obviously highly reliable and quite resistant to forces considerably in excess of those one normally encounters in everyday life This is not to say that failure will not occur merely that we were unable to produce it We desire to emphasize again however that these results related only to susceptibility of the prostheses to failure and to tissue response, *not* to the effect of these variables upon the *hearing* of the stapedectomized animal

We also believe that it is significant that no behavioral manifestations of vestibular damage could be observed Any penetration of the vestibule by *the prostheses might be expected to have produced immediate and observable* behavioral changes The fact that none were observed suggests that no penetration occurred.

Our results obviously do not support the findings of Andersen *et al* (1964) who reported disarticulation of the prostheses at "violent" levels. This is not surprising as their prosthesis was in an exposed dead temporal bone, while ours was in an intact living animal

DISCUSSION

Based upon the results presented above, we can see no real need to drastically restrict the duty *activities of stapedectomized persons after complete* recovery from initial surgery As always, common sense should be used but apparently the procedures are quite resistant to failure if successfully performed However as shown in Tables 1 and 2, original results of the insertion of the prostheses can be less than perfect, so that trauma could conceivably break down an initially imperfectly implanted prosthesis.

We could observe no significant basis for choice between the PE+V and SSP techniques. *If basis for choice exists, it is surgical, not mechanical*

It is appropriate to mention here that more research is necessary before we *can set exposure criteria for the patient's* work environment It will probably be some time before sufficient data are accumulated to enable us to evaluate the permanent effects of various noises upon the hearing of stapedectomized patients.

CONCLUSIONS

1 High intensity impulse noise and rapid changes in air pressure do not appear sufficient to cause significant prosthesis failure in stapedectomized monkeys.

2 Of the two variables tested, within the limits of this experiment, pressure changes appear to be more traumatic

3. No apparent difference appears to exist between the reliability of the stainless steel piston and the polyethylene and vein techniques

4 Solely on a basis of fear of prosthesis failure no reason can be seen for any drastic limitation of duty of stapedectomized patients.

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The uthors are grateful t Carl E. Guthrie for his v luable assistance in this study

ZUSAMMENFASSUNG

Zwecks Feststellung des Effektes von stossartigen Geräuschen und plötzlichen Druckveränderungen auf Patienten nach operativer Entfernung des Steigbügels wurden 40 Affen dieser Operation unterzogen und später Artilleriefeuer der plötzlichen Druckveränderungen in der Höhendruckkammer ausgesetzt. Es wurden zwei verschiedene Prothesen verwandt die Hälfte der Affen erhielt eine Polyäthy lenstütze und Adera fpropfung, die andere Hälfte einen Kolben aus rostfreiem Stahl. Nach Beendigung der Aussetzung wurden die Affen sofort durch Aussipegelung der Trommelf lle untersucht. Weder ein experimenteller Zerfall der Prothesen noch verhaltungsmässige Andeutungen vestibularer Pathologie wurden beobachtet. Es konnten keine wesentlichen Unterschiede zwischen den beiden angewandten Prothesen festgestellt werden. Auf Grund dieser Untersuchung besteht keine gültige Ursache für eine drastische Dienstbefreiung von P tienten nach Steigbügel chirurgie.

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THE ENDOCOCHLEAR POTENTIAL IN THE SHAKER 1 (sh-1/sh 1) MOUSE

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An endocochlear potential between 50 and 120 mV was found in ten deaf shaker 1 mice with atrophy of the organ of Corti and stria vascularis.

Lord & Gates, in 1929 first described the shaker 1 mouse as a new mutation of the house mouse (Mus musculus) They observed choreic movements of the head at birth or a few days afterwards, with a progressive development of the pattern to a typical form at 2 to 3 weeks of age Deafness developed within a few days, starting at the age of 22–30 days. The hereditary deafness in the shaker 1 (sh 1/sh 1) mouse was described as recessive heterozygotes being normal in behavior and retaining their sense of hearing throughout life. Gruneberg *et al* (1940) made observations on the structure development and cochlear microphonics of the inner ear of the shaker 1 The post foetal development of the inner ear appeared to pursue an identical course in both the normal and the shaker 1 mouse in the first 12 days of life Soon afterwards, degenerative changes were observed in the organ of Corti and stria vascularis of the shaker 1 Coincident with anatomical changes, the Preyer reflex was absent and the cochlear microphonics disappeared

Deol (1956) described the cochlea of the shaker 1 as being histologically normal at 3 weeks after birth, the age of onset of deafness. After the animals became deaf the hair cells began to shrink and their nuclei became pycnotic and migrated away from the basilar membrane The supporting cells lost their characteristic shape and the tectorial membrane curled up Visible degeneration of the stria vascularis occurred later than degeneration of the organ of Corti and its size as well as its histological structure were affected These anatomical and behavioral observations by Deol were confirmed by Mikaelian & Ruben (1964) and were correlated with changes in cochlear potentials and VIII nerve action potentials in the shaker 1 Cochlear potentials were first recorded from normal CBA-J mice on the eighth day after birth, but their appearance in the shaker 1 was delayed until the ninth day The range of cochlear microphonic responses in the shaker 1 was restricted and by the 21st day *post partum* the cochlear microphonics could no longer be recorded. The VIII nerve action potentials in the shaker 1 never attained the magnitude that it did in the normals and disappeared at 21 days of age

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Kikuchi & Hilding (1967) observed with the electron microscope degenerative changes in the stria vascularis of the shaker 1 Beginning at about 4 weeks of age, the marginal cells lost their vesicles and there was a reduction in their complex folds, endoplasmic reticula, ribosomes and Golgi complexes. At 7 weeks of age the intermediate cells became atrophic and the fluid space between cells increased

Since Békésy (1952) discovered the presence of a positive DC potential the endocochlear potential (EP) in the scala media of the guinea pig's cochlea, it has been shown that the stria vascularis is the most likely source of the EP (Davis *et al* 1958 Tasaki & Spyropolous, 1959)

In the present study the EP was measured in the normal adult CBA-J/CBA-J mouse and in the homozygous deaf adult shaker 1 mouse.

METHODS

Eleven normal adult CBA-J mice and ten adult shaker 1 mice were used All of the animals were six months or older The mice were anesthetized with 0 01 mg Thorazine and 0 03 mg Nembutal per gram of body weight The animals were held in a head holder The bulla was exposed through a post auricular incision and the basal turn of the cochlea was seen after making a hole in the latero-caudal aspect of the bulla. A Unigauge test reamer (no 0) was used to make a hole in the bony capsule of the cochlea and the surface of the underlying spiral ligament of the basal turn was exposed.

Glass capillary electrodes filled with 3 *M* KCl solution were used to record the EP The diameter of the tip was between 2 and 5 microns. A chloride-coated silver wire, 100 microns in diameter was inserted into the barrel of the capillary The indifferent electrode was placed in the neck muscles for all of the recordings. The DC output was fed into a cathode-follower Grass P-6 pre-amplifier and Grass polygraph (Model 5) Two of the shaker 1 mice were given lethal doses of Nembutal during DC recording in the scala media. The EP fell to zero as the animal s respiration failed.

Cochlear microphonics were recorded with a silver wire (0 002") that was inserted to the tip of a finely drawn glass capillary The electrode was placed in contact with the round window membrane The round window output was fed into the selective voltmeter input of a Hewlett Packard wave analyzer (Model 302A) and measured directly in microvolts, rms.

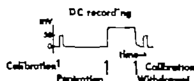


Fig 1 DC potential during penetration f capillary electrod thro gh the spiral ligament nd stria vasculari nd int the scala media f the basal turn f shaker 1 cochlea

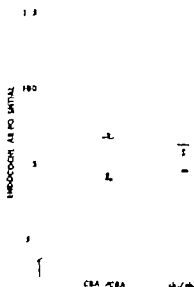


Fig 2. Distribution of values of endocochlear potential found in eleven CBA-J/CBA-J mice and ten shaker-1 mice Horizontal lines are mean values.

The experiments were carried out in an IAC sound proof room During recording of cochlear microphonics the ear was stimulated in a free field with pure tones 0 7 seconds in duration The tones were generated with the audio signal output of the wave analyzer (BFO) fed into a Grason Stadler electronic switch and interval timer attenuated with a Hewlett Packard attenuator (Model 350B) amplified with a MacIntosh 30 watt amplifier and led to a Janzen electrostatic speaker (Model 65) The sound pressure at the entrance to the external ear canal was measured with a calibrated Western Electric condenser microphone (Model 640AA)

The heads of the animals were removed, trimmed and processed for histology according to the procedure described by Ruben & Sidman (1967) The tissue was sectioned at 10 micra and stained with Toluidine blue Four of the shaker 1 cochleae were graphically reconstructed according to the method of Guild (1921) The remaining hair cells and the condition of the stria vascularis were mapped on the reconstruction One additional deaf shaker 1 mouse was given an injection of 10 $\mu c/g$ of Leucine H^3 with a specific activity of 2 C/mm This animal was sacrificed one hour later and prepared for radioauthography The sections were cut at 7 microns and the exposure time was 6 weeks. Kodak NB-11 emulsion diluted 1 1 with water at 37 C was used The slides were developed with Dektol developer at 19 C.

RESULTS

Electrophysiological observations

An EP between 54 0 mV and 109 0 mV with a mean of 83 9 mV was found in eleven normal CBA-J/CBA-J mice.

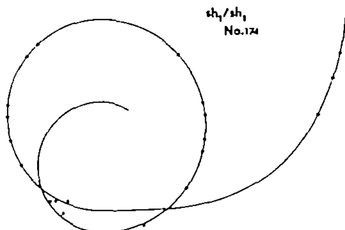


Fig 3 Graphic reconstruction of the cochlea of deaf dult mouse Dots, normally ppeari g hai cell st rs, segment of normally appeari g stria scul ris.

In ten deaf adult shaker 1 mice an EP between 50 0 mV and 120 0 mV with a mean of 81 7 mV was recorded from the scala media of the basal turn. Fig 1 demonstrates the DC recording from a capillary electrode inserted through the spiral ligament and stria vascularis, and into the scala media of the basal turn of a shaker 1 s cochlea. The distribution of the values of the EP for eleven CBA-J/CBA-J and ten shaker 1 is shown in Fig. 2.



Fig 4 Photomicrograph f the orga f Corti i the basal turn of a deaf adult shaker 1's cochlea.



Fig 5 Photomicrograph f the stria vascularis in the basal t rn f CBA-J CBA-J' cochlea (*right*) nd shaker 1 cochlea (*l ft*)

Four of the eleven shaker 1 mice were tested for a round window recorded cochlear response No cochlear microphonic was recorded above the 10 μV noise level of the recording system for frequencies between 1000 Hz and 20 000 Hz at intensities of 110 dB (relative to 0 0002 dynes/cm²) Normal input–output curves for the cochlear microphonics were obtained from five of the CBA-J/CBA-J mice tested.

Histological observations

Fig 3 shows a graphic reconstruction of the cochlea of a shaker 1 mouse with an endocochlear potential of 03.2 mV A few abnormal hair cells were found in each turn and the stria vascularis was atrophic throughout most of the cochlea Fig. 4 shows the atrophy of the hair cells in the basal turn of a shaker 1 mouse. Fig. 5 demonstrates the stria vascularis of a CBA-J/CBA-J on the right *and the atrophic stria from a similar portion of the* cochlea of a shaker 1 mouse on the left.

The radioaudiographic studies showed uptake of Leucine H³ in the spiral ligament, stria vascularis and spiral ganglion cells in the shaker 1 cochlea

DISCUSSION

The range of values for the EP in the CBA-J/CBA-J and the deaf shaker 1 are similar to those found in other mammals (Schmidt & Fernández, 1962) The normal EP in the shaker 1 indicates that this potential is not affected by the degenerative changes taking place in the cochlea of this animal Tasaki & Spyropolous (1959) found an almost normal EP in the adult waltzing guinea pig in which the organ of Corti had degenerated but in which there had been minimal degeneration of the stria vascularis. The potential in the guinea pig was reported to be highest close to the stria vascularis and Tasaki & Spyropolous felt that their results supported the hypothesis that the EP is generated by the stria vascularis.

Gruneberg *et al* (1940) described a progressive degeneration of the stria vascularis from base to apex in the shaker 1 Deol (1956) reported that the size as well as the histological structure of the stria in the shaker 1 showed degeneration. He described a loss of cytoplasmic processes of the marginal and basal cells of the stria and an increase in the vascularization. In the final stages large vacuoles appeared and in the extreme cases only the barest remnant of the stria remained.

The present study confirmed the previous observations that the stria vascularis in the shaker 1 atrophied. Despite these changes in the stria the EP was present in the deaf shaker 1 Suga *et al* (1964) found that the EP in the guinea pig could be maintained by segments of the stria vascularis. In addition the ability of the stria in the shaker 1 to take up Leucine H is evidence that this structure may be metabolically intact with regard to protein synthesis. It could be that a partially damaged stria vascularis can maintain the EP

RÉSUMÉ

U e potentielle endocochlée entre 60 et 120 mV était bservée dans 10 souris sourdes expérimentales (Shaker 1) avec trophie de l organ du Corti de stria vascularis.

ZUSAMMENFASSUNG

Ein endocochleares Potential zwischen 50 und 120 mV wurd an „zehn taubstummen shak 1 Mäusen gefunden mit iner Atrophie des Cortischen Org n u d der Stria vascularis.

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BINAURAL RECORD OF COCHLEAR POTENTIALS IN THE GUINEA PIG AND DIRECTIONAL HEARING

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Several experiments involving binaural records of cochlea potentials in the guinea-pig are described. The conductive properties of the two ears were studied as a function of frequency and intensity The variations of the responses were observed when the azimuth of the sound source was modified and also, in the case of bone-conducted t nes, when the point f application of the receiver on the cranium was changed. In another series of experiments, the possibl role of the ossicular muscles contrac ti s in directi nal hearing was investigated on wakeful animals.

Many mechanisms which are involved in binaural hearing, require a com plete identity of the auditory receptors, and particularly of their conductive properties. Such a similarity is very likely to occur owing to the bilateral symmetry of the skull and of the middle ear structures. In human beings, it is verified that the threshold of hearing is similar in both ears. Some small irregularities in the audiometric curves are only due, most likely to varia tions in drum mobility possibly through an ossicular muscle action This similarity has been demonstrated by Waetzmann (1935) and Langenbeck (1936) through systematic surveys. However the question may be raised as to what extent the electrical responses of the inner ear mainly the cochlear microphonics and action potentials, are equal on both sides, and how their variations reflect the properties of the sound field. For instance, it is possible that some slight changes of the ossicular chain impedance may provoke unnoticeable effects on sensitivity but may modify significantly the phase of the microphonics. On the other hand, it is possible that ossicular muscles tension exerts an active controle of the middle ear impedance Some experi mental results reported by Möller (1961) Simmons (1964) seem to indicate that such a control exists and that it is relatively independent on each side It could, possibly provide a sort of adaptation to the sound field and would play a role in directional hearing

The purpose of this paper is to describe some experiments which were performed in order to investigate the variations in the electrical responses of the two ears in various physiological and acoustical conditions.

Several questions have been particularly investigated. (1) The similarity of the responses from both sides on anesthetized animals. (2) The variations of the responses with the azimuth of the sound source (3) The variations

of the responses to bone conducted tones, according to the position of the receiver (4) The action of the ossicular muscles in binaural hearing on wakeful animals.

METHODS

The animal used was the guinea pig The cochlear potentials were recorded by means of a fine electrode made from steel wire varnished save at the tip (diam 100 μ) introduced in the bulla, and penetrating the scala tympani The operating technique was the same as that previously described (Legouix, 1957) It helped to record the potentials on anesthetized preparations as well as on wakeful animals. The anesthesia was provided by intraperitoneal injection of ethyl-urethane However when a quick awakening was required ether anesthesia was used In the case of chronic implantation, another silver wire was introduced under the skin to be used as an indifferent electrode. During the periods of rest, the wires were folded and fixed in a sort of belt attached around the neck It was verified that this method, which consists in introducing an electrode in the perilymph, has no detrimental effect on the cochlear responses, at least in the short term. Similar conclusions were reported recently by Simmons (1967) One advantage of this technique is that the electrical conditions of the record are better than with an electrode in contact with the round window membrane.

Similarity of the Responses from Both Ears in Anesthetized Animals

The animal was anesthetized with etyhl urethane, and fixed on a stereotaxic holder The head was held by means of two hollow bars penetrating slightly into the external ear canal The sound was introduced in these bars by two plastic tubes of the same size, and connected through a T connector to a compression chamber loud speaker This procedure helped to apply the same sound to the two ears. The recording electrodes were connected to twin amplifiers, associated with a two-beam oscilloscope.

The potential recorded by these electrodes is a mixture of the cochlear microphonics generated exclusively in the first coil of the cochlea, and of the whole nerve action potential (Tasaki & Fernàndez, 1952) The latter is due to the superposition of all the unit action potentials travelling in the auditory nerve fibers at the basal part of the cochlea However when the stimulus is a continuous sound or a pure tone, the action potential is almost unnoticeable. Its amplitude is then very weak owing to the desynchronisation of the individual action potentials. On the contrary when the stimulus is a brief sound a pip or a click, the summation in a short time of the activity of many units makes the whole nerve action potential appear relatively larger than the cochlear microphonics.

Cochlear microphonics

This potential follows closely the form of the sound wave and it could be verified that there exists a very good similarity of the responses from both

sides, at least for frequencies below 1500 Hz. Since the acoustical pressures are equal in the two ears, it may be inferred that the mechanical impedances are the same

For frequencies above 2000 Hz, some differences in amplitude and phase are observed in the responses of both sides. This indicates that the reactive factors of impedance are not entirely similar

When a complex sound is presented to the ears, some differences between the waveforms of the microphonics of both sides are observed. It may be related to the inequalities of the higher components. In the same way at low frequencies and at high pressures levels, some harmonics appear and distort the waveform in different manners in the two sides.

Action potentials

The action potential of the auditory nerve is recorded by the same electrode and is easily observed when the stimulus is a brief sound like a pip or a click. In this case the electrode records the cochlear microphonics corresponding to the stimulus, followed, after a short delay by the nervous response constituted by the three classical deflexions N_1 N_2 and N_3

When the stimulus is the same click, on both sides, the action potentials show a good similarity (Fig 1) in amplitude as well as in the waveforms. It happens sometimes that two slightly different clicks provoke nervous deflexions which are apparently similar

Changes in the Responses according to the Position of the Sound Source

The perception of the position of the sound source depends to a great extent upon the differences in intensity and in time delay of the stimuli which arrive at the two ears. These differences result from the characteristics of the sound field and from the geometrical properties of the auditory receptors, mainly the size and the shape of the pinna (Batteau, 1968) the distance between the two tympanic membranes, and to some extent the diffraction produced by the body and the head.

Role of the external ear

The anatomical structure of the external ear provides, by itself a differential sensitivity according to the position of the source In order to study this particularity the animal was set upon an horizontal board, made of absorbing material, and a sound source, constituted by a loud speaker was placed in different positions, but at the same distance (30 cm) of the tympanic membrane of one of the ears. The opening of the loudspeaker whose diameter was 1.5 cm, could be considered as punctual In these conditions, the differences of the responses could be related to the orientation effect of the external ear structures and mainly of the pinna.

It was observed that the amplitude of the responses is not equal in all positions (Fig 2) and that the sensitivity is greater when the source is



Fig 1.

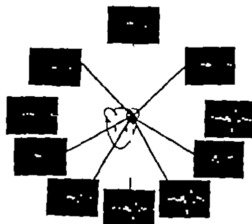


Fig 2

Fig 1 Simil rity f th microphonics nd action potential f the auditory nerves picked up i both cochle m. Microphonics \ nd \ the two cl ssical d flexion f the ction potential (The stimul i a cli k.)

Fig 2 Changes in the size f the cochlea respo se to a click (microph ics and action pote tial) ccording t the position f the sound so rce The distance from th tympa ic m mbrane is kept constant (30 cm)

located in front of the animal in a direction at 45 relative to the longitudinal axis of the body This corresponds, in fact, to the orientation of the pinna at rest. In a similar manner a minimum of sensitivity appears when the source is located in an opposite direction, that is to say behind the pinna.

The role of the pinna may be demonstrated directly When the source s in the position of the maximum of sensitivity the cochlear responses are reduced in a significant manner when it is pushed back against the cranium All these effects are greater at the high frequencies when the wavelength is closed to the dimensions of the pinna.

However at frequencies above 3000 Hz the amplitude of the responses is a very irregular function of the position of the source as it is already known (Firestone, 1930 Nordlund, 1962) and the same response may be obtained in several positions

Differences in the cochlear responses of the two ears according to the azimuth of the sound source

The same experimental setting was used but the distance from the loud speaker relative to the middle of the line joining the two ears was maintained constant In these conditions, the cochlear microphonics, produced by a pure sound varies, in amplitude and phase according to the azimuth of the source When the stimulus is a pip or a click, it is possible to measure the delay of the cochlear microphonics corresponding to the time required for the sound to travel from the source to the receptor In the particular case of

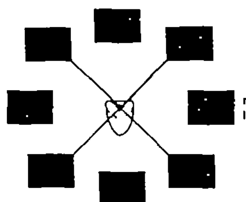


Fig 3.

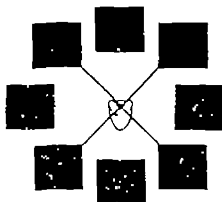


Fig 4

Fig 3 Changes in the cochlear microphonic response from the two cochleae when the sound source is moved around the head; the distance to the sound source is constant. Frequency 3000 Kc.p.s.

Fig 4 Changes in the amplitude and the latency of the action potential of the auditory nerve according to the position of the sound source. The distance from the middle of the line joining the two ears to the loud speaker is kept constant. The stimulus is a pip

a small animal like the guinea pig, whose inter-aural distance is about 1.5 cm, the differences of time of arrival do not exceed 0.3 ms.

When the action potential provoked by a click is recorded from both sides, it is observed that the latency of the action potential increases with the distance from the source in a greater extent than the delay of the cochlear microphonics (Figs. 3 and 4). This results, as is well known, from the fact that the intensity is decreased with the distance and that the latency of the action potential becomes correlatively larger. Hence, the cumulative effect of the lowered intensity and of the delay of the stimulus arriving at the ear enlarges the time differences of the nervous messages in the central pathways (Fig. 5).

Changes in the Cochlear Responses provoked by a Bone Conducted Stimulus according to the Position of the Receiver

According to the classical works, two main mechanisms may occur in bone conduction. At low frequencies (below 800 Hz) a translation mechanism is prominent. The various parts of the cranium vibrate in phase but the ossicles, thanks to their inertia, tend to lag behind the cranium, so that the ossicles show displacements relative to the cochlea, as it occurs in air conduction. At the higher frequencies (above 800 Hz) the various parts of the cranium show phase lags in such a manner that compressions may occur in some regions, particularly in the cochlear walls. For these reasons, the compression mechanism would become more prominent.

At low frequencies, when the translatory mechanism occurs, the responses

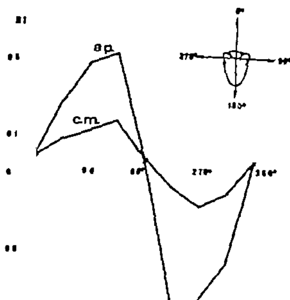


Fig 5 Variation f the del y f the cochlear microphonics and f the latency of the ction potential when the sound source is moved round the head. The com l tire effect f the time d l y of the sound nd of the change in int nsity mak larger the riati s of the lat ncy f the action potenti l

from the two ears should present a complete similarity since the stimulus is the same. However this equality is not verified when the impedance of the middle ear is modified (Legouix & Tarab, 1959 Tonndorf *et al* 1960) Some differences in phase and amplitude also occur when the position of the bone-conduction receiver is modified as it appears in the following observations.

In these experiments, the head of the animal was maintained in a stereotaxic holder by means of hollow ear bars. This procedure modifies slightly the impedance of the cranium but in a symmetrical manner which has no bearing upon the results. A classical bone-conduction receiver with an electrostatic shield was used for the stimulation

The results showed that the microphonic responses provoked by low frequency sounds present significant differences according to the point of application of the bone conducted stimulus on the cranium For instance when it was in the medial plane in contact with the upper foreteeth th microphonic potentials were identical in phase and in amplitude On the contrary when the receiver was in contact with the lateral part of th cranium in the temporal region the microphonic responses were opposite in phase At intermediate positions, various phase relationships were observed between 0 and 180

These phase relationships are explained by the symmetrical arrangement of the ears (Fig 6) When the receiver is applied in the medial plane of the cranium, the components which act upon the ossicles on both sides are symmetrical relatively to the medial plane so that the tympanic displacements

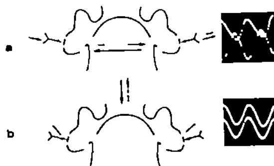


Fig 6 V riations f the phase relationship f the cochlea microphonic potenti l f the two ears, provoked by bon -conducted sound, ccording t the point f pplication of the bone conduction recei er These variation are well explai ed by the translatory mechanism f stim lation.

relative to the cochlea, are in the same direction in the two ears. On the contrary when the receiver is applied in the temporal region, the ossicular displacements, relative to the cochlea, are in opposite directions.

Bárány showed (1938) on the human ear that the response to a bone-conducted sound (low frequency) varied in intensity and phase according to the location of the receiver on the cranium This was demonstrated by a method of cancellation of the bone conducted tone by an air conducted tone whose phase and intensity was properly adjusted The intensity of the sensa tion was maximum when the receiver was touching the temporal region, in the axis of the ossicular chain. The phase was shifted by 180 when the receiver was applied on the other side These facts, as well as the results presented above, appear as direct evidences for the translatory mechanism of bone conduction.

It was also possible to demonstrate by the same method, that slight variations of the impedance of the middle ear or of the acoustic properties of the ear canal, were able to provoke great changes in amplitude and in phase of the responses, as it was already described (Legouix & Tarab, 1959 Tonndorf *et al* 1966)

For frequencies above 1000 Hz the phase relation between the responses of the two ears appears to vary in an irregular manner In the higher range of frequencies, as is known, the wavelength is shorter than the dimensions of the cranium. This explains how the two ears may be in different vibratory state.

Role of the Ossicular Muscles in Binaural Hearing

The role of the ossicular muscles in hearing has not yet been completely elucidated. Several theories have been proposed. First, the ossicular muscle contractions, by restraining the amplitude of movement of the ossicles would protect the cochlea against the loudest sounds. Some experimental evidence for this mechanism has been presented (Galambos & Rupert 19 9) How

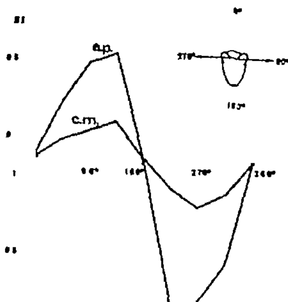


Fig 5 Variations of the delay f the cochl microphonics and f the l tency of the action potential, when the sound source i moved around the head. The cumul tive effect f the time delay f the sound and f the change in intensity mak l rger the variation f the latency f th action pot ntial.

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These phase relationships are explained by the symmetrical arrangement of the ears (Fig 6) When the receiver is applied in the medial plane of the cranium, the components which act upon the ossicles on both sides are symmetrical relatively to the medial plane, so that the tympanic displacements,

animals, as compared with that observed on anesthetized preparations, is their somewhat smaller amplitude This difference may be easily noted at the beginning of anesthesia, when a progressive increase of the potentials occurs (Fig 7) (Legouix & Moulonguet, 1957) In the same way as it was shown by Simmons, the relationship between the amplitude of the microphonics and the acoustical pressure is altered These changes are easily related to the decrease of the ossicular muscle tension

Another feature of the microphonic response in the wakeful animal is the rapid variations of its amplitude. These small modulations (which were described by Simmons) in cats are also observed in guinea-pigs. They occur at rates between 2–4/sec, but their amplitude does not exceed 1 dB. Their perceptive significance is not clear

Apart from modulations appear some slower variations of amplitude which may last several seconds but are no more than one or 2 dB. Sometimes they seem to appear randomly but they may be provoked by a short acoustic stimulus like a click (Fig. 8)

These variations are bilateral, but they are often larger in one side. Sometimes, the changes in amplitude of the microphonics are very reduced and can hardly be measured on the oscilloscope However some activity of the ossicular muscles may be detected by observing the action potential provoked by a click This type of response is very sensitive to any alteration of the stimulus, and they show in the wakeful animal large and rapid variations, which are related to the ossicular muscle contractions, since they disappear after cutting these muscles. These variations are a very sensitive test of their constant activity

In order to obtain more precise information on the possible action of the ossicular muscles in directional hearing, the cochlear microphonics were recorded in the two ears and applied to the two deviation plates of the oscilloscope, to form Lissajou s pictures. This procedure appeared to be very convenient to observe the rapid changes of phase and amplitude, particularly in normal living conditions. Unfortunately it was difficult to provoke significative reactions of the guinea-pig

However it appeared from the observations, that the strength of the ossicular muscle contractions showed differences in both sides in several instances, although it was not possible to relate the changes to particular positions of the sound source. This independent control of the muscles of the two ears is, anyway an important fact in favor of their participation in directional hearing

DISCUSSION AND CONCLUSIONS

The mechanisms which are involved in directional hearing raise neurophysiological and also physical problems. One of the latter is the role of the geometrical characteristics of the receptor organs, mainly the pinna and the external ear canal

The results of the observations which are presented above which are relative to the electrical responses of the two cochleae show that the ears of the guinea pig are not much different from artificial stereophonic receptors, like, for instance, those which were used by Nordlund (1962) in the study of directional hearing Like these receptors, guinea-pig ears show a very good similarity of sensitivity and phase at least in the lower range of frequencies and in resting conditions

However several features are particular to the guinea pig structures. One of these is the relatively short distance which exists between the two ears. This factor in principle, should decrease the sensitivity to localize sound sources. Anyhow some neurophysiological mechanisms may compensate this unfavorable parameter One example of such a mechanism is found in the cumulative effect of the intensity and the delay of arrival of the stimulus, on the latency of the auditory nerve action potential

Another important problem in directional hearing is the possibility of a central control on the receptors. Such a control might be exerted through the action of the ossicular muscles or directly on the sensitivity of the sensory cells or of the auditory fibers. Several results, such as those reported by Simmons (1964) concerning the microphonic potential and the myographic record of the activity of the ossicular muscles, are in favor of the hypothesis of their participation in the auditory behavior

On the other hand, a separate nervous control of the muscles of each side would provide, as suggested by Lawrence (1965) a mechanism for the adaptation of the receptors to the sound field The observations, reported in this paper relative to the bilateral record of the cochlear potentials from the two ears, do show that a separate control exists, although there is a correlation in the contractions of both sides. In the same way it was demonstrated in human beings, that changes in impedance due to the contractions of the ossicular muscles, are not equal in the two ears, but are somehow stronger in the stimulated ear if the sound is applied on one side (Moller 1961) However in spite of such observations, it is difficult to attribute a precise role to the ossicular muscle in directional hearing The fact that the tensions are stronger on the side which receives the larger intensity suggest that the change of impedance which follows, would tend to reestablish the equality of the stimulation in the two ears. It would act in the same way as the motion of a subject who turns the head to face the sound source Such action would agree with the demonstrated fact that the angular sensitivity is greater in a narrow space limited by an angle of 20 in front of the head

The second hypothesis is the possibility of a direct control on the receptor sensitivity through the action of efferent pathways. The recent works concerning the electrical activity of the olivo-cochlear fibers (Galambos, 1960 Fex, 1962 Desmedt, 1962) have shown that the electrical stimulation of the crossed and direct olivo-cochlear fibers provokes an inhibition of the action potential of the auditory nerve However no observation has clearly demonstrated in wakeful mammals, during normal behavior the occurrence of such

inhibitions (Legoulx, 1957 Galambos, 1960) while in the hen, Gernushi *et al* (1960) reported inhibitions in some circumstances like pecking corn In that respect, it is interesting to note that the observations presented in this paper on wakeful guinea-pigs, do not show any evidence for an inhibi tory action in normal behavior nor any neural interactions between the two auditory nerves, which might have an influence on directional hearing

RÉSUMÉ

Plusieurs séries d expériences concernant l'enregistrement bilatéral des poten tiels cochléaires chez le cobaye so t décrites dans cet article. La similitude des propriétés d conduction des deux oreilles a été vérifiée pour diverses fréquences et intensités. Les variations des réponses en foncti n de l'azimut de la source sonore ont été étudiées ainsi que dans le cas de sons transmis par voie osseuse en fonction de la position du point d' pplication du vibrateur sur le crâne Dans une autre série d'expériences, le rôle possible des contractions des muscles ossi culaires dans l'orient tion auditive a été recherché sur des animaux éveillés.

ZUSAMMENFASSUNG

Mehrere Versuchsreihen, die die beiderseitige Aufzeichnung des Potentials in der Cochlea beim Meerschweinchen betreffen, werden im vorliegenden Artikel beschrieben. Die Gleichheit der Schallübertragungsfähigkeit beider Ohren wurde auf erschieden Frequenzen und Lautstärken geprüft. Di Schwankungen der A tworten wurd n je nach Azimuth der Tonquelle untersucht, sowie, bei knochen-übermitt lten Tönen, nach Lage des Anwendungspunktes des Vibrators uf dem Schädel. In i er anderen Versuchsreihe wurde die mögliche R lle der Spannung der Mitt lohrmusk ln in bezug uf Richtungshören bei erwachsenen Tieren unter sucht.

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THE TONE DECAY TEST

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The purpose of this investigation was to estimate (1) The correlation between tone decay degree and topographical diagnosis, age and pure tone threshold. (2) The significance of applying different test frequencies. A total of 2123 ears were tested in 500, 2000 and 4000 Hz by means of a manual method. A strict correlation was found, on the one side between a high degree of tone decay and cerebello-pontine angle tumours and on the other side a low degree or missing tone decay in normal hearing ears. In six diagnostic groups the perstimulatory threshold shift was unequally distributed among the different test frequencies. The age factor does not influence the tone decay noticeably while the degree of hearing loss in some cases seems to be an important factor. Two test frequencies are recommended, 500 and 2000 Hz.

The threshold tone decay test has been the object of much interest in recent years because of its clinical application in the diagnostic differentiation of sensori-neural hearing losses. Mainly two different procedures have been elaborated, the manual method which is accomplished by means of a continuous stimulus tone, the intensity of which is adjusted to the threshold in 5 dB steps, and the selfrecording fixed frequency technique (Békésy audiometry) by means of continuously changing intensities spaced between the just noticeable and the just not noticeable level. The tone decay is the amount the intensity must be increased to keep the tone audible. Sörensen (1962) and Palva *et al.* (1967) have recorded tone decay by means of the manual method, both authors in relatively large case materials, the former using a single test frequency (2000 Hz) and the latter partly single, partly various frequencies, however, without more definite information about the frequencies employed. Flottorp (1964) studied tone decay at several frequencies, but on a limited number of ears only. Palva *et al.* (1967) and Palva & Palva (1966) on the basis of two different investigations stated that the manual technique is more sensitive than the selfrecording one.

The aim of this investigation was: (1) By means of manual tone decay test to investigate further the correlation between the tone decay degree and factors such as the topographical diagnosis, age and pure tone threshold. (2) To study if the perstimulatory changes at threshold were equally distributed in different test frequencies (500 Hz, 2000 Hz and 4000 Hz) and if not, whether divergencies should be correlated to the factors mentioned

above In that case the utilization of different test frequencies might perhaps establish a safer basis for diagnostical evaluations.

METHOD

The threshold shift was judged by means of the manual procedure using general diagnostic audiometers (Peters SPD 5 and Amplivox 83) After determination of pure tone thresholds for interrupted stimuli in the frequency range 125 Hz–8000 Hz, continuous tone in the frequencies 500 Hz, 2000 Hz and 4000 Hz were presented at a 5 dB sensation level The subject had been instructed to listen and to keep his hand up as long as the tone was heard and let it down if the tone faded away If the tone disappeared within 60 seconds, the intensity was increased by 5 dB and the procedure was repeated until the tone could be heard at the same intensity level for 60 seconds, or until the maximum output of the audiometer In order to classify the degree of the threshold shift, the results in this material were differentiated in the following types

Type I *a* No tone decay beyond 5 dB relative starting intensity
Type I *b* A tone decay of 10 dB
Type II The tone intensity reached within 3 minutes a plateau at (*a*) 15–20 dB above the initial intensity (*b*) 25 dB or more above the initial intensity
Type III A continuous threshold drift within 3 minutes and a tone decay after 2 minutes exposure of (*a*) 20 dB or less, (*b*) 25–35 dB (*c*) 40 dB or more

Especially in some cases of marked hearing losses, the maximum output of the audiometer did not permit the tone to be heard for a sufficiently long time to determine the tone decay type according to the above-mentioned definitions. In such cases the tone decay type was defined from the mean gradient expressed in dB per minute as shown in Table 1

Table 1 *Determination of the tone decay type in cases of limited time course*

Tone decay gradient dB min	Tone decay type classification
2.4 and less	I
2.5–4.9	I b
5.0–7.4	II
7.5–9.9	II b
10.0–14.9	III
15.0–19.9	III b
20.0 and more	III

SUBJECTS

The case material comprises 2123 ears from about 1800 subjects. The patients were partly hospitalized, partly attending the outpatient ENT department during the years 1963–67 All patients were submitted to routine ENT examination pure tone and speech audiometry and tone decay test Complete investigations were performed in selected cases in accordance with the conventional diagnostical lines of the ENT department radiography eventually tomography of the temporal bone pneumoencephalography angiography neurological examination electroencephalography and other laboratory examinations. Most patients with cochlear or retrocochlear lesions were subjected to otoneurological examination including postular and caloric vestibular tests, Fowler test, stapedius reflex test some cases to Békésy audiometry and electronystagmography as well. All cases classified as retrocochlear lesions were examinated during the stay at the neurological department, and the diagnosis has in most cases been verified during the subsequent operation or after autopsy The subgroup "cerebello-pontine angle tumours" comprises acoustic neurinoma 10 glioma 3, subarachnoid

Table 2. *Age and sex distribution of the diagnostic groups (number of ears)*

Diagnostic group		Age in years										
		0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99	Sum
1	Male	0	22	28	62	90	61	23	2	0	0	288
	Female	2	28	47	86	135	146	72	10	1	0	527
	T tal	2	50	75	148	225	207	95	12	1	0	815
2	Male	0	0	0	0	10	80	143	53	0	0	286
	Female	0	0	0	0	16	66	136	86	15	1	320
	T tal	0	0	0	0	26	146	279	139	15	1	606
3	Male	0	4	1	7	18	31	24	7	0	0	92
	Female	0	3	6	3	13	27	29	13	0	0	94
	Total	0	7	7	10	31	58	53	20	0	0	186
4	Male	0	2	20	33	61	107	76	9	0	0	308
	Female	0	3	6	27	23	39	52	12	0	0	162
	T tal	0	5	26	60	84	146	128	21	0	0	470
5 A	Male	0	0	0	0	3	0	2	0	0	0	5
	Female	0	0	0	0	0	6	2	1	0	0	9
	T tal	0	0	0	0	3	6	4	1	0	0	14
5 B	Male	0	0	0	0	4	0	2	2	0	0	8
	Female	0	0	0	3	8	8	5	0	0	0	24
	Total	0	0	0	3	12	8	7	2	0	0	32
Sum	Male	0	28	49	102	186	279	270	73	0	0	987
	Female	2	34	59	119	195	292	296	122	16	1	1136
	Total	2	62	108	221	381	571	566	195	16	1	2123

cyst 1 All patients except one (with acoustic neurinoma) are operated on The subgroup other expansive intracranial diseases are labelled as follows tumour cerebri 20 arterovenous aneurysma 2, intracranial haematoma 2 cerebral abscess 2 Only in one patient the lesion was located to the temporal lobe

The case material was classified in the following groups and subgroups, according to the anatomical location or the aetiology of relevant diseases

Group 1 Normal hearing ears, defined as hearing losses of 20 dB or less in the frequency range 500–4000 Hz without any known ear lesion

Group 2 Presbyacusis, i e hearing losses of 25 dB or more at one or more of the frequencies 500 2000 and 4000 Hz due to ageing, and without any other disease or injury that could reasonably be the cause of the hearing loss.

Group 3 Conductive hearing losses

Group 4 Hearing losses of cochlear origin (sensory hearing losses)

Group 5 Retrocochlear lesions. (A) Cerebello-pontine angle tumours. (B) Other expansive intracranial diseases (without hearing loss)

The numbers of ears in each of the groups and subgroups are shown in Table 2.

RESULTS

The results are shown in Tables 3–8. In the normal hearing group of 815 ears serving as a reference (Table 3) no tone decay could be pointed out in the test frequency 500 Hz, every case belonging to the type I *a* In the test

Table 3 *Graduation of tone decay as a function of age*

Group 1 Normal hearing ears. (815)

Age (years)	Test frequency 500 Hz Tone decay type I	Test frequency 2000 Hz Tone decay type I	I b	Test frequency 4000 Hz Tone decay type I	I b	II
0–9	2	2		2		
10–19	50	49	1	50		
20–29	73	71	1	71	1	3
30–39	148	147	1	142	5	1
40–49	225	210	8	201	13	8
50–59	207	200	7	291	9	7
60–69	93	93	2	77	9	8
70–79	12	12		11	1	
80–89	1	1		1		
Sum ears	815	797	18	748	39	28
Per cent	100	98	2	92	5	3

Table 4 *Tone decay graduation as a function of age and hearing loss*

Group 2 Presbyacusis. ($n = 606$.)

	Test frequency 500 Hz	Test frequency 2000 Hz						Test frequency 4000 Hz						
	Tone decay type	Ton decay type						Tone decay type						
	Ia	I	Ib	II	IIb	IIIa	IIIb	I	Ib	II	IIb	III	IIIb	III
Age (years)														
40–49	26	25	1					22	4					
50–59	148	124	15	8	1			103	19	20	5	1		
60–69	278	238	18	16	3	2	1	173	44	28	18	5	1	9
70–79	138	105	14	10	2	4	3	87	21	10	3	5	4	8
80–89	15	8	3	2	1	1		7	4	2	0	1	1	
90–99	1	1						1						
Hearing loss (dB)														
20 and less	429	219	13	5				19	3	0	2			
25–40	143	207	26	16	4	2		191	39	22	13	3	1	
45–60	27	66	7	14	2	5	2	127	32	26	8	2	2	6
65 and more	7	9	5	1	1	0	2	63	18	12	3	7	3	11
Sum ears	606	501	51	36	7	7	4	383	92	60	26	12	6	17
Per cent	100	83	8	6	1	1	1	65	15	10	4	2	1	3

frequencies 2000 and 4000 Hz 2% and 5% respectively had a slight threshold drift of 10 dB. In the frequency 4000 Hz 3% had a "plateau" tone decay at 15–20 dB. No cases of type III could be demonstrated in the normal hearing group. As to the age factor no significant correlation between increasing age and threshold drift was traceable.

The age itself therefore seems not to influence the tone decay noticeably.

In the presbyacusis group (Table 4) a some more frequent tone decay is demonstrable in the frequencies 2000 and 4000 Hz, the type II amounting to 7 and 14% and the type III 2 and 6% respectively. The tone decay increases with increasing hearing loss at the same frequencies, while the age factor still seems to be less deciding. Altogether the type I is predominant and in the test frequency 500 Hz no tone decay was found, 100% of the ears being referred to the type I a, in spite of the fact that some of the patients were at a great age and some had a high degree hearing loss.

The groups of conductive and sensory hearing loss were each originally divided in aetiological subgroups. The "conductive" cases had the following diagnostical headings: Sequelae otitidis (141 ears), chronic otitis (20 ears), otosclerosis (7 ears), other non suppurative middle ear lesions (18 ears).

The cochlear subgroups were: Menière's disease (178 ears), occupational hearing loss (161 ears), congenital and early acquired impairments (70

Table 5 Graduation of tone decay as a function of age and hearing loss

Group 3 Conductive hearing loss. (n = 186.)

	Test frequency 500 Hz Tone decay type		Test frequency 2000 Hz Tone decay type				Test frequency 4000 Hz Tone decay type						
	Ia	Ib	Ia	Ib	IIa	IIb	Ia	Ib	II	IIb	IIIa	IIIb	IIIc
Age (years)													
10–19	7		7				0	0	1				
20–29	6	1	5	2			6	1					
30–39	10		10				8	1	0	1			
40–49	31		31				21	4	2	1			
50–59	58		57	1			51	5	1	0	0	0	1
60–69	53		51	1	0	1	36	12	1	1			
0–79	20		18	2			10	2	0	0	1	1	
Hearing los (dB)													
20 nd less	72		60	1			11	4					
25–40	65		68	1			74	10	1	2			
45–60	31		35	3	0	1	39	5	3				
65 a d more	17	1	18	1			23	6	2	1	1	1	1
Sum ears	185	1	179	0	0	1	147	25	8	3	1	1	1
Per cent	100	<1	96	3	0	<1	79	13	4	2	<1	<1	<1

ears) head injuries (38 ears) sudden deafness (15 ears) hearing loss of luetic origin (3 ears) other cochlear lesions (5 ears)

These subgroups were however not significantly deviating from the main groups as to the distribution of tone decay types. The two main groups therefore are presented without subdivisions (Tables 5 and 6)

In the "conductive" group of 186 ears more than 90% had a tone decay type I in all the test frequencies. In the frequency 500 Hz all the ears turned out to be type I in the frequency 2000 Hz all but one belonged to the same type even some high degree losses and some cases of advanced age The type III tone decay was recorded in the frequency 4000 Hz only and here in 3 ears of 186

In the group cochlear hearing losses (Table 6) as well, at the test frequency 500 Hz all the cases were gathered within the type I tone decay However this main group differs from the previous groups by a more pronounced types II and III in the frequencies 2000 and 4000 Hz, the type II amounting to 11 and 1 and the type III to 2 and 11% respectively

The 5 A–B groups of VIII nerve and cerebral disorders total 46 cases with the following subdivisions (A) 14 cases, (B) 32 cases.

In the 5 A group of cerebello-pontine angle tumours (Table 7) the results

Table 6 *Tone decay graduation as a function of age and hearing loss*

Group 4 End organ hearing loss. (=470.)

	Test frequency 500 Hz Tone decay type		Test frequency 2000 Hz T decay type						Test frequency 4000 H Tone decay type						
	I	Ib	I	Ib	IIa	IIb	III	IIIb	I	Ib	II	IIb	III	IIIb	III
Age (years)															
10–19	4	1	4	1					4	0	0	1			
20–29	26		21	1	4				16	6	2	1	0	0	1
30–39	60		54	2	2	1	1		37	9	5	6	1	2	
40–49	83	1	73	7	2	0	2		61	9	4	2	3	2	
50–59	145	1	117	12	11	3	1	2	92	18	15	7	7	4	3
60–69	127	1	85	16	21	3	3		59	14	18	12	11	7	7
70–79	21		16	1	3	0	0	1	10	1	4	3	2	0	1
Hearing loss (dB)															
20 and less	246		164	5	2				22	2	3	2			
25–40	95	1	99	6	10	2	3		95	16	8	8	4	1	
45–60	81	1	77	20	22	4	1	2	100	25	20	15	7	4	2
65 and more	44	2	30	9	9	1	3	1	65	14	17	7	13	10	10
Sum ears	466	4	370	40	43	7	7	3	282	57	48	32	24	15	12
Per cent	99	1	79	9	9	2	2	<1	60	12	10	7	5	3	3

Table 7 *Tone decay pattern as a function of age and hearing loss*

Group 5A. Cerebello-pontine angle t mours. (n = 14)

	Test frequency 500 Hz Tone decay type							Test frequency 2000 H Tone decay type		Test frequency 4000 Hz Tone decay type	
	I	Ib	II	IIb	III	IIIb	III	IIIb	III	IIIb	III
Age (years)											
40–49	1	0	0	0	0	1	1		3		3
50–59	1	0	1	0	0	0	4	1	5	1	5
60–69	1	1	0	0	0	0	2		4		4
70–79	0	0	0	0	0	0	1		1		1
Hearing loss (dB)											
20 and less	2								2		
25–40	1	0	0	0	0	1	2		1		
45–60	0	1	0	0	0	0	3	1	2	1	4
65 and more	0	0	1	0	0	0	3		8		9
Sum ears	3	1	1	0	0	1	8	1	13	1	13

Table 8 *Tone decay pattern as a function of age*

Group 5 B Other intracranial expansive processes that do not affect the conventional threshold of hearing. (*n* = 32)

	Test frequency 500 Hz Tone decay type	Test frequency 2000 Hz Ton decay type			Test frequency 4000 Hz Tone decay type				
	Ia	Ia	Ib	IIa	Ia	Ib	IIa	IIb	IIIa
Age (years)									
30–39	3	3			2				
40–49	12	11	1		10	0	1	0	1
50–59	8	6	1	1	5	2	1		
60–69	7	5	0	2	1	0	5	1	
70–79	2	2			2				
Sum ears	32	27	2	3	21	2	7	1	1

are unambiguous and on that account significant in spite of the small numbers. At the test frequencies 2000 and 4000 Hz tone decay type III were recorded in all the 14 cases and at the test frequency 500 Hz in more than the half We feel that the test frequency 500 Hz in most cases is influenced at a later development of the disease as compared to the higher frequencies.

In the subgroup 5 B (other expansive intracranial processes which do not affect the conventional threshold of hearing) the results do not differ significantly from those in normal group and the presbyacusis group (Table 8)

DISCUSSION

The most dominant feature of this investigation is the relatively strict correlation between a high degree tone decay and angle tumours as opposed to the low degree or missing tone decay in the normal hearing ears. This confirms previous observations of other authors.

It is further evident from our results that the perstimulatory threshold shift is quite unequally distributed among the different test frequencies.

Figure 1 illustrates the relative distribution of the tone decay types II and III at the test frequencies 500, 2000 and 4000 Hz among the six different diagnostic groups. The columns indicate an increasing incidence f types II and III decay at the test frequency 4000 Hz, when starting from the normal hearing group and going through the group of conductive deafness, cerebral expansive processes without hearing loss, presbyacusis, end organ hypacusis to the cerebello-pontine angle tumours. The columns of the test frequency 2000 Hz show a similar increasing incidence of types II and III for the four last diagnostic groups, while a significant threshold hift at 500 Hz is present in the cerebello-pontine angle tumour group only (10 out of 14 cases)

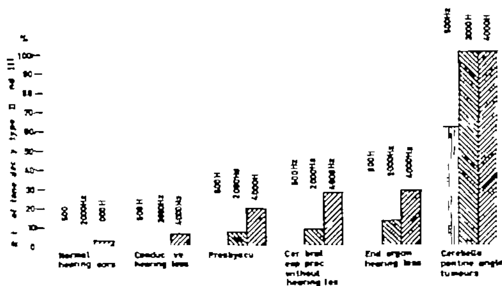


Fig 1 Tone decay pattern for each test frequency (500, 2000 nd 4000 Hz) functio of diagnostic group.

The tone decay test thus seems to be a valuable test as to the diagnostic separation of VIII nerve disease but not in the differentiation between the other groups. A type III tone decay in all the three test frequencies, included 500 Hz, definitely suggests an expansive angle tumour At 4 000 Hz a considerable perstimulatory threshold shift may be present in all our diag nostic groups. This test frequency therefore has little diagnostic value and could possibly be dropped to simplify the audiometric examination. Although a pathological outcome at the test frequency 2000 Hz may be found in all the diagnostic groups except the normal hearing group and the group of conductive hearing losses, the results in the angle tumour group differ distinctly from the other ones. We therefore will recommend the tone decay test to be carried out at two frequencies, i.e. 500 Hz and 2000 Hz. The application of the lower test frequency however should be necessary only in cases with significant tone decay at the higher frequency

We could find no significant indication that the age itself in any of the diagnostic groups seems to influence the tone decay noticeably The degree of hearing loss on the other side seems to be an important factor both in the presbyacusis group and in the group of end organ hearing losses (sensory hearing losses) It must be stressed, however that reliable test results to a great extent depends upon satisfactory precautions concerning apparatus, test procedure, cooperation etc.

CONCLUSIONS

The threshold tone decay test is a valuable diagnostic aid to decide the presence of cerebello-pontine angle tumours. The application of two test frequencies (500 and 2000 Hz) is recommended.

The degree of hearing loss seems to play a role for the tone decay pattern in both the presbyacusis group and the group of cochlear hearing loss while the age itself does not influence the tone decay in any diagnostic group

ZUSAMMENFASSUNG

Die Absicht dieser Arbeit war einerseits die Beziehung zwischen dem Tonhörschwellenschwund und der topografischen Diagnose anderseits den Einfluss von Alter und Tonhörschwelle zu untersuchen. Ausserdem wünschten wir eine Antwort auf die Frage zu geben, ob es von diagnostischer Bedeutung wäre beim Höradaptionstest mehrere Frequenzen anzuwenden. 2123 Ohren wurden in den Frequenzen 500 2000 und 4000 Hz durch die manuelle Methode geprüft. Die Untersuchungsserie wurde in sechs diagnostische Gruppen geteilt. Die Ergebnisse haben bestätigt, dass Fälle mit Kleinhirnbrückenwinkeltumoren einen ausgesprochenen *Hörschwellenschwund zeigen, während bei Normalhörenden keine oder nur* leichte Schwellenerhöhung beobachtet wird. Im übrigen scheint die Probe von wenig differentialdiagnostischer Bedeutung zwischen perzeptiven Schwerhörigkeitsgruppen zu sein Das Alter an sich hat keinen Einfluss auf die Grösse des Schwellenschwundes, sondern es scheint, als ob eine schlechte Hörschwelle in vielen Fällen einen grösseren Schwellenschwund verursache Es wird empfohlen die Testfrequenzen 500 und 2000 Hz anzuwenden.

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FINE STRUCTURE OF THE OTOLITHIC MEMBRANE IN THE SQUIRREL MONKEY

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The zonal structures of the otolithic membrane in the squirrel monkey were investigated by electron microscopy and the different morphology of the different zones was described. The definite existence of the cupular zone and subcupular zone was determined in the subhuman primates and some functional considerations were made. The cupular zone was divided into two subzones which demonstrated different morphological features. The structure of the otoconia was also investigated electron-microscopically.

The macula-otolithic membrane is considered to be a mechanoreceptor of gravitoinertial forces; therefore, it is very important to know the architectural arrangement of the otolithic membrane which exists between statoconia and the sensory hair cells. This structure is, however, very fragile and difficult to preserve for both light and electron microscopic investigations. The purpose of this study is to add some recently obtained electron microscopic information in subhuman primates to the classic description of the submicroscopic level.

MATERIAL AND PROCEDURE

Squirrel monkeys were anesthetized by the intraperitoneal injection of sodium pentobarbital (30 mg/kg). A postauricular atticotomy approach was utilized to expose the incudo-stapedial area. After removing the malleus and incus, the stapes was removed with a fine pick. Cold 5% glutaraldehyde solution buffered with cacodylate (pH 7.4) was gently injected into the vestibule. A fine curette was used to remove the facial canal and nerve so that the macula utriculi could be better exposed. The cold glutaraldehyde solution injection was repeated and a fine excavator was used to expose the horizontal and the superior semicircular canal ampulla areas. The macula utriculi and the ampullae of the horizontal and superior semicircular canals were dissected out with a fine pick. Thereafter the specimens were immersed in cold 5% glutaraldehyde solution buffered with cacodylate at

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pH 7 4 for further fixation The specimens were fixed for 2 hours at 4 C (Sabatini *et al.*, 1963) After multiple changes of buffered solution during the subsequent 48 hours, the tissue was post fixed in cold cacodylate-buffered 2% osmium tetraoxide solution for 1 hour Thereafter the specimens were rapidly dehydrated through a graded series of ethanol solutions, and then embedded in a mixture of Maraglas and D.E.R 732 (Erlandson 1964)

One-micron sections stained with modified methylene blue-azure II and counterstained with basic fuchsin were made for light microscopic orientation (Richardson *et al.*, 1960) Some of the one-micron sections were stained in periodic acid Schiff (PAS)

Ultrathin sections were stained in 0.5% uranyl acetate (Wolfe *et al* 1962) followed by 1% lead citrate (Venable & Coggeshall 1965) The sections were examined by RCA EMU-3H electron microscope operated at 100 kv

The 20 micron thick celloidin sections of the normal squirrel monkey macula, which were originally prepared for the purpose of other investigations, were stained in alcian blue for this investigation.

FINDINGS

Light microscopic findings

The 1 micron section stained in periodic acid-Schiff clearly demonstrated the zonal architecture of the otolithic membrane (Fig 1) The upper part of the cupular zone was most densely stained in PAS, while the other part of the cupular zone was lightly stained The subcupular zone was much less stained in PAS. The otolithic zone was stained moderately The findings suggested the probable existence of neutral mucopolysaccharide in the cupular zone

The 20-micron thick celloidin sections (Fig 2) which were stained in alcian-blue exhibited the existence of acid mucopolysaccharide in the cupular zone of the otolithic membrane the maculae and the area of the basement membrane

Electron microscopic findings

The electron microscopic findings (Fig 3) demonstrated that the area around the ciliae was most clear in other words less electron dense while the electron density was slightly more towards the microvilli of the supporting cells. Thus, the subcupular zone is not a smooth and flat zone The so-called subcupular zone was clearly distinguishable from the cupular zone

As determined by the electron micrographs, the cupular zone was divided into two different subzones. The cupular zone 2 (CZ 2) which is closely located to the subcupular zone was found as slightly electron dense This electron density was diffusely distributed The next was the cupular zone 1 (CZ 1) In this zone the electron density was not as evenly distributed as in the cupular zone-2 There were more electron dense areas which looked like



Fig 1 One-micron section of the squirrel monkey utricula macula stained I periodic acid-Schiff The zonal rch tect re l clearly distinguishable. Both f the ot lithic zone nd the cup l zone were w ll preserved in thi partical section and the hexagonal shaped otolith were bserved. Not some difference i the PAS density i the cup l zone 480



Fig 2 A iew from the alcian-blue st ined 20-micron section f the squirrel monkey macula (fixed i Heidenhai Susa solutio) Not the cupula zone, macula, nd the basement membrane rea were densely stained, which is suggesting the probabl existence of acid mucopolysaccharide. 510

pH 7.4 for further fixation. The specimens were fixed for 2 hours at 4 C (Sabatini *et al.*, 1963). After multiple changes of buffered solution during the subsequent 48 hours, the tissue was post fixed in cold cacodylate-buffered 2% osmium tetraoxide solution for 1 hour. Thereafter the specimens were rapidly dehydrated through a graded series of ethanol solutions and then embedded in a mixture of Maraglas and D.E.R. 732 (Erlandson, 1964).

One-micron sections stained with modified methylene blue-azure II and counterstained with basic fuchsin were made for light microscopic orientation (Richardson *et al.*, 1960). Some of the one-micron sections were stained in periodic acid Schiff (PAS).

Ultrathin sections were stained in 0.5% uranyl acetate (Wolfe *et al.*, 1962) followed by 1% lead citrate (Venable & Coggeshall, 1965). The sections were examined by RCA EMU-3H electron microscope operated at 100 Kv.

The 20 micron thick celloidin sections of the normal squirrel monkey macula, which were originally prepared for the purpose of other investigations, were stained in alcian blue for this investigation.

FINDINGS

Light microscopic findings

The 1 micron section stained in periodic acid Schiff clearly demonstrated the zonal architecture of the otolithic membrane (Fig. 1). The upper part of the cupular zone was most densely stained in PAS, while the other part of the cupular zone was lightly stained. The subcupular zone was much less stained in PAS. The otolithic zone was stained moderately. The findings suggested the probable existence of neutral mucopolysaccharide in the cupular zone.

The 20-micron thick celloidin sections (Fig. 2) which were stained in alcian blue exhibited the existence of acid mucopolysaccharide in the cupular zone of the otolithic membrane, the maculae, and the area of the basement membrane.

Electron microscopic findings

The electron microscopic findings (Fig. 3) demonstrated that the area around the ciliae was most clear, in other words less electron dense, while the electron density was slightly more towards the microvilli of the supporting cells. Thus, the subcupular zone is not a smooth and flat zone. This so-called subcupular zone was clearly distinguishable from the cupular zone.

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Fig. 1 One-micron section f the sq irrel monkey utricula macula stained i periodic cid-Schiff. The zonal rchitect re i learl distinguishabl. Both f the ot lithic ne nd the cupul one were well preserved in thi particula section nd the hexag nal shaped ot lith were bserved. Not some difference i the PAS density i the cupular zone. ×160.



Fig. 2. A iew from the lcian-blue stained 20-micron section of the squirrel monkey macula (fi ed i Heidenhai Sum solutio). Not the cupular zone, macula, nd th basement membrane rea were densely stained, which is suggesting the probabl existence f cid mucopolysaccharide. ×10.

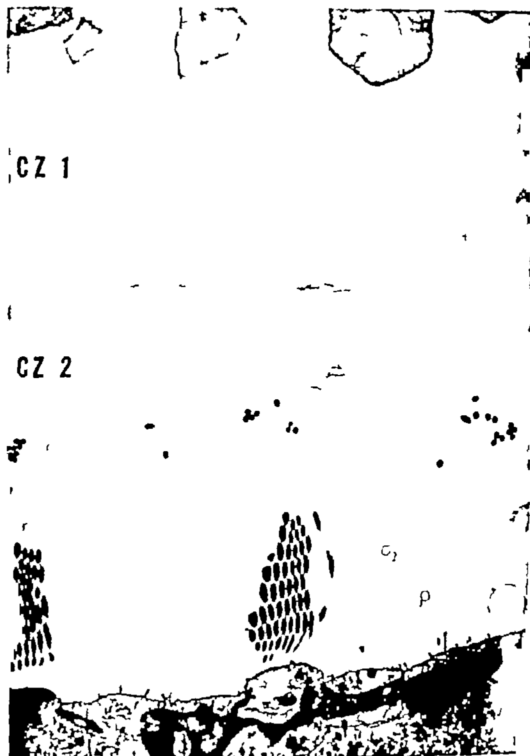


Fig 3. Electron microg ph d mo strates the diff rent zone f the otolithi membrane N t th les lectro den ity i the bcup l ne nd much densely t ined rod sh ped rea in th pper part f the cup l r zone *CZ 1* C p l r a 1 *CZ 2* cup l zone-2, described th t t 6300



Fig 4 Electro micrograph which w stained by colloidal iron solution (Carra f l 1965) demonstrates different distribution of the colloidal iron particles. The distribution pattern f the colloidal iron w fairly identical t the distribution f the lectro density 7600.



Fig 5 Electron micrograph d mon trates the squirrel m nk y otoliths. Not the he gonal shaped ot lith which were partl rrounded by the exten i f the cupula which ha moderat lectron density 12,000

rods when the macula was cut in the perpendicular plane (Figs. 3, 4) Around this rod-shaped area there were slightly electron dense areas like the cupular zone-2. The lower end of these rod shaped areas extended into the cupular zone-2 therefore round shaped much more electron dense areas were observed in some parts of the cupular zone-2 (Fig 4) Thus, the landmark between the cupular zone-1 and the cupular zone-2 was not generally so clear However these rod shaped areas were more pronounced in the cupular zone 1 while moderately dense diffuse areas were found more in the cupular zone-2.

The hexagonal shaped otoliths were seen even in ultrathin sections (Fig 5) The edges of the otoliths were relatively smooth but the size of the otoliths was quite varied According to the sectioning plane quite a number of different shapes of the otoliths were demonstrated. In most of the otoliths the electron density was generally evenly distributed

Around some of the otoliths there was the extension of the cupular zone which showed moderate electron density In addition, in some parts of the otolithic zone, the otoliths were attached to the rod shaped area of the cupular zone-1

According to the present investigation in the squirrel monkey there was definitely some distance between the tips of the ciliae and the otoliths and these two structures were not found to be directly connected.

DISCUSSION

The zonal architecture of the otolithic membrane in the subhuman primate was studied electron microscopically Although other schematic illustrations of the otolith end organ did not describe a zonal structure (Wersäll & Lundquist, 1966) one of us has previously described different zonal structures of the primate otolithic membrane (Igarashi, 1967) These electron micrographs support this conclusion

The different electron density between cupular zone and subcupular zone was demonstrated in the present investigation, and, the existence of the subcupular zone (Vilstrup, 1950 Wersäll, 1968) which was previously described in many light microscopic investigations, has been confirmed. The contents of this subcupular zone is not known. In addition, it is still not definitely known that this space freely opens to the endolymphatic space or not. If it does not, the contents would not be the endolymph.

As described in findings, two subzones of the cupular zone were suggested namely diffusely dense cupular zone-2 and rod-shaped cupular zone-1 (Fig. 6) The separation of these two zones was not quite clear-cut and rod shaped structures penetrated into cupular zone 2, close to the tips of sensory ciliae. These rod shaped structures were previously described as filaments or some times as possible extensions of the ciliae when viewed with light microscopy Tenaglia (1925) investigated unfixed material by dark field illumination technique and described submicroscopic granules and filaments. The struc

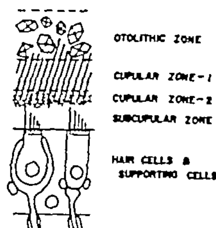


Fig 6 Schematic drawi g demon trates different z nal structure in the cro secti n of the ot lith nd org n. The l dmarks between these different zonal structures are usually not q ite distinguishabl

ture (filament) was subsequently described in the bird (Brock, 1926 Wer ner 1960) in the guinea pig (Eckert Möbius, 1926 Wittmaack, 1956) in the rabbit (Werner 1960 Wittmaack, 1956) in the cat (Eckert Möbius, 1926) in the dog (Wittmaack, 1956) in the squirrel monkey (Igarashi, 1966) and in the man (Johnsson & Hawkins, 1967)

The authors have also investigated cat otolith end organs by using identi cal preparation procedures, but have failed to find clear rod shaped struc tures electron microscopically however the difference in the electron den sity between the two subzones in the cat was also considerably clear

As is seen both in thick (1 micron) and in ultrathin sections, the direction of these rods was quite unique as was previously described at light micro-scopic level In addition the connection between rod shaped cupular zone-1 and otolithic zone was not likely to be very strong as was exhibited partic ularly in 1 micron section The rod shaped structures definitely reached to the statoconia however the statoconia were not firmly embedded in the cupular zone itself in other words, the statoconia could be separated from the cupular zone This observation has been made in primates previou ly (Igarashi, 1966) After the special stainings of the otolithic membrane Wis-locki & Ladman *(1954 1955) have suggested the existence of mucopoly* saccharide and glycoprotein in the otolithic membrane

The hexagonal crystal otoconia was cut in thin secti ns without any decal cification (this is probably fr m the identical hardness of the otoconia and the embedding media) and investigated electron microscopically The ize of statoconia varied very much as described by Carlstr m *et al* (1953 Carl strom & Engstrom (1955) Werner (1960) Engstrom *et al* (1966) and Iurato (1967)

Iurato (1967) has demonstrated a direct attachment between ciliae and *otoliths in the rat specimen* however this was not observed in our present

investigation of subhuman primates. The existence of the cupular zone in the squirrel monkeys suggests there are attenuation and generalization effects from gravitoinertial forces which act on statoconia On the other hand, the direct attachment of sensory hairs and statoconia in lower animals is a well known fact (Brock, 1926)

ACKNOWLEDGMENTS

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RÉSUMÉ

Nous avons exami é au microscope électronique les structures zonales de la membrane otolithique dans *Saimiri sciureus* et décrit la morphologie différent aux zones différentes. Nous avons déterminé l existence des zones cupulaire et sous-cupulaire dans les primats presque humains et les avons considérés du point de *vue fonctionnel. Nous avons divisé la zone cupulaire en deux sous-zones qui* montrent des traits morphologiques différents. Nous avons examiné également au microscope électronique la structure de l' toconie

ZUSAMMENFASSUNG

Mit Hilfe der Elektronenmikroskopie wurden di zonalen Strukturen der Ot lithmembran im *Saimiri sciureus* (squirrel monkey) untersucht, und die andersartige Morph logie der verschiedenen Zon n wurde beschrieben. Es wurde die zwei fellose Existenz der kupularen Zon sowie der subkupularen Zone in der subhumanen Primaten bestimmt und einige funktionale Betrachtungen gemacht. Die kupulare Zone wurde in zwei U terz en, di verschiede morph logische For men zeigten, geteilt. Die Struktur der Otoconia wurde benfalls elektronenmikroskopisch untersucht.

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PASSIVE ION TRANSPORT THROUGH THE REISSNER MEMBRANE

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The influence of the alteration of the passive ion transport through the Reissner membrane on endocochlear potentials (EP) was investigated in 24 guinea pigs. Simultaneously the influence of these alterations on the cochlear microphonic (CM) was determined It was found that the substitution of the anion Cl by SO in the scala vestibuli did not cause any changes of EP and CM, which means that the movement of Cl ions through Reissner's membrane does not contribute substantially to creating the membrane potentials. The equilibration of the K and Na concentration gradient on both sides of the Reissner membrane transitorily enhanced the EP value by +6.87 mV and reversibly reduced the CM by 40 to 60% The equilibration of the Na and K concentrations on both sides of the Reissner membrane and the blocking of the active ion transport reduced the EP to +0.88 mV ±1.07 which is a reduction lesser by 14 4 mV than during the mere blocking of the active ion transport maintaining the concentration gradient on both sides of the Reissner membrane The voltage difference thus formed represents the approximate value of the membrane potential of the Reissner membrane. The equilibration of the ion concentration together with the blocking of the active ion transport caused a transitory reduction of CM by 85 to 95%

New information about the transport of ions through cell membranes facilitate the investigation of transport processes in the inner ear The ion transport through cell membranes has two forms active and passive.

The active transport was characterized by Ussing as a process transporting substances in cells against an electrochemical gradient. This transport represents a manifestation of cell activity and depends on their undisturbed metabolism. If some blocking substances, e.g cyanides, 2,4-dinitrophenol, iodoacetate, interfere into the cell metabolism, the active transport of ions is stopped, which manifests itself by an alteration of the potential generated by it

The passive ion transport consists in their motion within the tissue medium which is caused by various physical factors, as e.g by a concentration or electric gradient, ion mobility and the mechanical structure of the membrane acting as a barrier preventing free ion diffusion.

The quantity of the intracellular potential is determined by the resistance of the membrane and by the concentration difference especially of monovalent ions It follows from this fact that an alteration of the ion concentra

tion on one side must lead to a change of the membrane potential of these cells.

In the previous paper an active ion transport was documented in Reissner membrane. In the present paper the influence of the absence of some ion in the scala vestibuli or of an alteration of its concentration on the passive transport through the Reissner membrane was investigated by means of electrophysiological methods.

Smith *et al.* (1954) established that there is a high potassium concentration (144.4 milliequivalents-meq/l) and a low Na concentration (15.8 meq/l) in the endolymph, whereas the perilymph has an opposite composition—Na 150.3 meq/l, K 4.8 meq/l. Further, the chlorine amount in the endolymph was determined to be 107.1 meq/l, whereas in the perilymph it amounts to 121.5 meq/l. Later on these findings were complemented by Citron, Exley according to Fernández (1967) by the finding that the concentration of Ca and Mg is almost identical on both sides of the Reissner membrane (Ca 3.0 meq/l, Mg 2.0 meq/l).

To be able to establish the passive ion transport through the Reissner membrane and its membrane potential, the ion concentration of the perilymph was altered. The perilymph was substituted by a modified solution without any chlorides and by a fluid with the same ion composition as that of the endolymph, while the active ion transport was simultaneously blocked by means of 2,4-dinitrophenol, as well as without this blocking.

METHODS

Twenty-four healthy guinea pigs of an average weight of 250–300 g, anesthetized by means of 20% urethane were used in the experiments. The endocochlear potentials (EP) and the cochlear microphonic (CM) were measured by means of glass microelectrodes introduced into the second turn through the stria vascularis into the scala media. The glass microelectrodes were filled with 3 *M* KCL and connected by a chlorinated Ag wire across a cathode follower with a straight current amplifier. The amplified cochlear potential were controlled by an oscilloscope and simultaneously registered by a recorder throughout the experiment. To establish the activity of hair cells short sounds (1 sec) of 500, 1000 and 4000 Hz of an intensity of 60 dB were used.

The fluid was exchanged by means of a thin cannula introduced into the aperture in the basal turn of the scala vestibuli. A second aperture was drilled in the apex of the bone cochlea; through this aperture the fluid flowed into the bulla and from this it was sucked off, cf. the diagram (Fig. 1).

The experimental animals were separated into four groups. In the first (control) group normal Ringer solution was used for the perfusion of the scala vestibuli.

In the second group a modified Ringer solution was used: the chloride (Cl) ions were substituted by sulfate ions (SO_4) in an equivalent amount (Na_2SO_4 75 m*M*, $KHCO_3$ 2.6 m*M*, $CaSO_4$ 1m*M*).

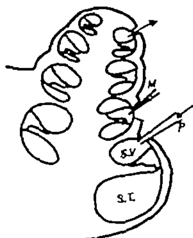


Fig. 1. Perfusion diagram of the scala vestibuli. S.T scala tympani; S.V scala vestibuli; P perfusion cannula inserted into the scala vestibuli; M microelectrode inserted into the scala media of the second cochlear turn.

In the third group a solution of approximately the same ion composition as that of the endolymph an "artificial endolymph" was used (K 144.4 meq/l, Na 15.8 meq/l, Cl 110 meq/l, Ca 3.0 meq/l, Mg 2.0 meq/l).

In the fourth group the "artificial endolymph" with 2,4-dinitrophenol in a concentration of $1 \cdot 10^{-3}$ M was used for perfusion.

For a statistical evaluation of EP and CM their original values after opening the scala vestibuli before the onset of perfusion were determined. The scala vestibuli was perfused between the 10th and 15th minute of the experiment. All values were registered within an interval of 30 sec. For the statistical evaluation the values obtained in 5-minute intervals and between the 10th and 25th minute in 1 minute intervals were applied.

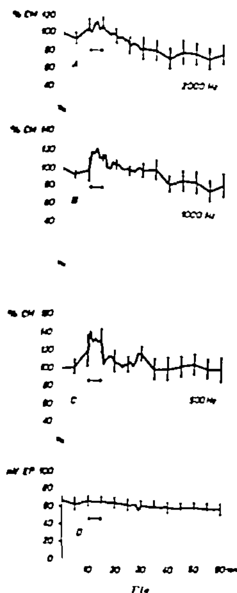
With respect to the biological dispersion of the measured values all CM were calculated as a percentage to facilitate their comparison, the EP values were left in absolute values for a better estimation of alterations around zero (shift from positive to negative values). The presented graphs were constructed according to the arithmetic mean and the standard deviation from the average.

RESULTS

In the first (control) group (scala vestibuli perfused by Ringer solution between the 10th and 15th minutes) the results are presented in Fig. 2, where graph A shows the response of CM to a sound of 2000 Hz in per cent, graph B the response of CM in per cent to 1000 Hz, graph C—the response of CM in per cent to 500 Hz and graph D—EP in mV.

It is evident that the rinsing did not influence the CM corresponding to sounds of 2000 Hz (graph A). The response to 1000 and 500 Hz was actually enhanced throughout the perfusion (B, C). The perfusion did not influence EP in any evident way (D).

In the second group (perfusion of scala vestibuli between the 10th and 15th minutes by means of the modified Ringer solution, where Cl ions were



Fig

Fig 3.

Fig 2 I fluence f the perf l f scal estib li b mean f Ri ger solution on the EP nd MP ⟷, Perf l Interv l f scala v stibuli; I st nd rd deviation from me lues.

Fig 3 Infl ce f perf l f scala estibuli b mean modified Ri ger soluti with Cl io bstituted by SO ions on EP nd MP ⟷, Perfusi Int rv l f scal ti buli I st ndard deviatio from mea l es.

substituted by SO) the results are given in Fig 3, where graph A h w the response of CM in per cent to a tone of 2000 Hz, graph B the re ponse of CM in per cent to 1000 Hz and graph C the resp nse of CM in per cent to a tone f 500 Hz respectively and graph D—the response of EI in mV

The perfu i n of the scala vestib uli did not influence the CM corre pond ing to the tones f 2000 and 1000 Hz (graph A, B) During the perfu i n only the CM values increased correspon ling to the tone of 500 Hz (graph C

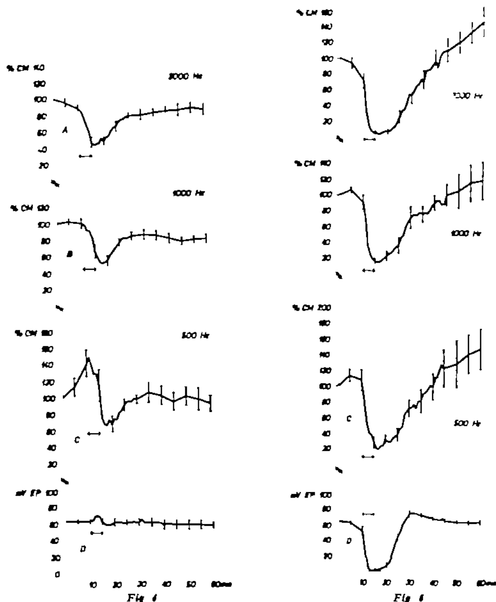


Fig 4 Influence of scala vestibuli perfusion by means f "artificial ndolymph on EP nd MP ⟷, Perf ion interval f scala vestibuli I standard deviati from mea lues.

Fig 5 Infl ence of perfusi f scala estibuli by means f "artificial endolymph with 2,4-dinitrophenol EP nd MP ⟷, Perfusion interval f scala vestibuli; I, standard deviation from mean alues.

The perfusion by this solution did not influence to any visible degree the EP values (graph D)

In the third group (perfusion of scala vestibuli between the 10th and 15th minutes by a solution of "artificial endolymph") the results are presented in Fig. 4 where graph A shows the response of CM in per cent to a sound of

2 000 Hz, graph B—that to 1000 Hz, graph C—to 500 Hz and D the response of EP in mV

In this case perfusion caused a rather rapid drop of the MP values. The greatest drop was observed in the response to 2000 Hz (graph A) where in the 16th minute the CM values decreased to 43 4 — 3 0 a somewhat lesser decrease to 51.0 ± 6.25 was established in the 18th minute to tones of 1000 Hz (graph B) and the least drop to 66 7 ± 0.9* was found at 500 Hz in the 18th minute. From the 16th to the 18th minute all CM values observed began to rise again and from the 30th minute on to the end of the experiment their course did not differ from that of the control group.

The perfusion simultaneously caused at first a small increase of EP to 70.81 mV ± 1.89 i.e by 6.87 mV (graph D) however already after an interval of a 3-minute perfusion these values began to decrease again, which lasted to the 17th minute when they amounted to 59.50 mV ± 3 40 and afterwards there was an increase almost to the original values of 63 0 mV where they stayed to the end of the experiment

In the fourth group (perfusion of scala vestibuli between the 10th and 15th minutes by "artificial endolymph" solution combined with 2,4-dinitrophenol) *the results are shown in Fig 5 Graph A presents the responses* of CM in per cent to a tone of 2000 Hz, graph B that to 1000 Hz, graph C the response of CM in per cent to 500 Hz and graph D shows the response of EP in mV

The perfusion caused a rapid decrease of CM values in response to all *tones used with the most significant drop occurring at the 10th minute. The* response of CM to sounds of 2000 Hz decreased from the initial 100 to 4.81 ± 1 11 (graph A) those to sounds of 1000 Hz to 14 92 ± 1.8 (graph B) and the responses to 500 Hz to 19.35* ± 3 68 (graph C). From the 16th minute when there was a maximum decrease of values, they began to increase slowly and they reached the original values between the 30th and 50th minutes (graphs A, B, C) The perfusion caused simultaneously a rapid drop of EP so that in the 13th minute EP values decreased to 0.88 mV 1 0 They remained at this value to the 17th minute afterwards they began to increase slowly at the onset rapidly later on, so that in the 28th minute the original values were restored and remained constant to the end of the experiment (graph D)

The cannula was adjusted to the perfusion aperture in the basal turn for the perfusion of the scala vestibuli and closed the perilymphatic space. The propagation of the sound into the apex during perfusion was thus improved and the response to the frequencies perceived at the apex therefore increased (especially that to 500 Hz

DISCUSSION

The passive ion transport always occurs from a higher electrochemical potential to lower ones. The K and Na concentrations of different order in the scala media and the scala vestibuli condition the existence of a m m

A lealo-l rp § 61

brane potential on the Reissner membrane The permeability of the Reissner membrane for ions was demonstrated by Rauch & Köstlin (1962 and 1963) who established the passage of labeled ^{42}K and ^{24}Na ions from the scala vestibuli to the scala media Evidence for the permeability of the Reissner membrane is brought also by the electronoptic findings of Lawrence *et al* (1961) Iurato (1967) Duvall & Rhodes (1967)

In the previous paper of the author the active ion transport through the Reissner membrane was proved by electrophysiological methods (Prażma, 1969) Among other findings, it was shown that after an efficient blocking by means of 2,4-dinitrophenol applied to the scala vestibuli, negative EP values appear (-13.6 ± 1.02 mV) This value represents part of the membrane potential caused by the concentration difference between scala media and scala vestibuli and the resistance of the Reissner membrane acting against the diffusion of single ions, or respectively between the scala media and the blood plasma in the stria vascularis. In somewhat different experimental conditions—at the anoxia of the whole animal—Butler (1965) Johnstone (1965) and Konishi *et al* (1967) measured negative EP values -30 to -40 mV The difference between the values found by the above authors and our results mentioned above is caused by various experimental conditions. Probably the lower values -30 to -40 mV correspond to the actual state as all cells coating the ductus cochlearis were excluded from any activity by the total anoxia of the animal. In our experiment only the active transport in the cells of the Reissner membrane and in those of its immediate surroundings were blocked The remaining cells coating the walls of the cochlear duct, especially the lower part of the stria vascularis, were not affected by this intervention, therefore their active ion transport was conserved and a positive EP value arose

Evidence of the passive transport through the Reissner membrane was demonstrated in the second, third and fourth experimental groups. In the second group of experiments, as has already been mentioned, the transport of Cl ions was investigated in such a way that these ions were substituted in the perfusion solution by SO_4 ions thus a different concentration gradient was caused between the endolymph and the perilymph in the scala vestibuli. With respect to the fact that the EP values were unaltered, it can be assumed that Cl ions either did not penetrate through the Reissner membrane or that SO ions passed in the same way as Cl ions.

In the third group of experiments the perilymph in the scala vestibuli was substituted by the "artificial endolymph" without any disturbance of the active ion transport After the equilibration of the concentration differences on both sides of the Reissner membrane a short term increase of EP by $+6.89$ mV was observed This increase could be interpreted as follows the higher K concentration in the perilymphatic space enhanced for short period (of three minutes) the transport of potassium ions from the scala vestibuli into the scala media, which manifested itself by an increase of the EP value During the subsequent ten minutes EP values slowly returned to the initial

2 000 Hz, graph B—that to 1000 Hz, graph C—to 500 Hz and D the response of EP in mV

In this case perfusion caused a rather rapid drop of the MP values. The greatest drop was observed in the response to 2000 Hz (graph A) where in the 16th minute the CM values decreased to 43.4% — 3.0 a somewhat lesser decrease to 51.0 ± 6.?% was established in the 18th minute to tones of 1000 Hz (graph B) and the least drop to 66.? ± 9.0% was found at 500 Hz in the 18th minute. From the 16th to the 18th minute all CM values observed began to rise again and from the 30th minute on to the end of the experiment their course did not differ from that of the control group.

The perfusion simultaneously caused at first a small increase of EP to 70.81 mV ± 1.89 i.e. by 6.8? mV (graph D) however already after an interval of a 3-minute perfusion these values began to decrease again, which lasted to the 17th minute when they amounted to 59.?0 mV ± 3.49 and afterwards there was an increase almost to the original values of 63.0 mV where they stayed to the end of the experiment.

In the fourth group (perfusion of scala vestibuli between the 10th and 15th minutes by *"artificial endolymph" solution combined with 2,4-dini-*trophenol) the results are shown in Fig. 5. Graph A presents the responses of CM in per cent to a tone of 2000 Hz, graph B that to 1000 Hz, graph C the response of CM in per cent to 500 Hz and graph D shows the response of EP in mV.

The perfusion caused a rapid decrease of CM values in response to all tones used with the most significant drop occurring at the 16th minute. The response of CM to sounds of 2000 Hz decreased from the initial 100 to 4.81 ± 1.11 (graph A) those to sounds of 1000 Hz to 14.02 ± 1.8 (graph B) and the responses to 500 Hz to 10.3?% ± 3.68 (graph C). From the 16th minute when there was a maximum decrease of values, they began to increase slowly and they reached the original values between the 39th and 59th minutes (graphs A B C). The perfusion caused simultaneously a rapid drop of EP so that in the 13th minute EP values decreased to 0.88 mV 1.07. They remained at this value to the 17th minute afterwards they began to increase slowly at the onset rapidly later on so that in the 28th minute the original values were restored and remained constant to the end of the *experiment (graph D).*

The cannula was adjusted to the perfusion aperture in the basal turn for the perfusion of the scala vestibuli and closed the perilymphatic space the propagation of the sound into the apex during perfusion was thus improved and the response to the frequencies perceived at the apex therefore increased (especially that to 500 Hz.

DISCUSSION

The passive ion transport always occurs from a higher electrochemical potential to lower ones. The K and Na concentrations of different order in *the scala media and the scala vestibuli condition the existence of a mem*

tiale (EP) untersucht. Zugleich wurde der Einfluss dieser Veränderungen auf die Mikrophonpotentiale (CM) verfolgt.

Es wurde festgestellt, dass die Ersetzung des Cl-Anions durch ein SO_4-Anion in der Scala vestibuli keine Veränderungen des EP und CM hervorrief was bedeutet, dass die Bewegung der Cl-Ionen durch die Reissnersche Membran nicht wesentlich zur Bildung des Membranpotentials beiträgt.

Ein Ausgleich der K und Na-I nenk nzentrationen beiderseits der Reissner schen Membran steigerte das EP vorübergehend um +6,87 mV und das CM wurde umg kehrt um 40 bis 60% herabgesetzt.

Ein Ausgleich der K und Na-I nenk nzentrationen u beiden Seiten der Reissnerschen Membran und die Blockierung des aktiven Ionentransportes setzt das EP auf +0 88 mV ±1,07 herab, was eine um 14.4 mV geringere Senkung vorstellt als bei der blossen Blockierung des akti en Ionentransportes mit Aufrechterhaltung der Konzentrationsdifferen en zu beiden Seiten der Reissnerschen Membran. Der entstanden Spannungsunterschied stellt den ungefähren Wert des Membranpotentials der Reissnerschen Membrane dar Der Ausgleich der Ionenkonzentrationen gemeinsam mit der Blockierung des akti en Ionentransportes verursachte eine vorübergehende Senkung des CM und 85 bis 95%

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TYMPANOSCLEROSIS

Electron Microscopic Study

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A light-microscopic study revealed the proliferation of collagen fibers, hyaline degeneration and dark blue in HE-staining in the sclerotic masses. At the above-mentioned site the following findings are obtained through an electron microscope: (1) Degenerating mitochondria with electron-dense masses in fibrocyte-like cell. (2) Electron-dense cell stroma, so-called autophaged lysosome and membrane-bound fragments in fibrocyte-like cells or among the collagen bundles. (3) Close to or near the cell membrane numerous collagen fibers with electron-dense material. This fact was regarded as newly produced fibers in fibroblast after calcification has taken place. (4) By X-ray microanalysis in electron microscope these electron-dense materials were proved as calcium-phosphate crystals—probably apatite clusters. (5) X-ray microanalysis showed that intracellular electron-dense material contained more calcium than phosphate, whereas extracellular electron-dense plaque contained more phosphate than calcium. From this we may conclude that it is uncertain whether hypercalcinosis is in the extracellular fluid. (6) Various other substances Fe, Cl, Cu, Mg, Cr, Mn. ( ) The shapes of apatite clusters are doughnut-like, of the form of an annual ring, or tortoise-shell pattern. (8) Animal examination showed that hyperparathyroidism caused calcification of tympanosclerosis.

Zöllner (1955, 1956, 1963) described the pathogenesis, diagnosis and special surgical management of tympanosclerosis. Since then tympanosclerosis has attracted great attention from otologists concerned with improvements through reconstructive microsurgery of the middle ear: Shambaugh (1959), Fuzisawa (1962), Goodhill (1960), House & Sheehy (1960), Harris & Weiss (1961), Claninger (1962), Sheehy & House (1962), Joseph & Gordon (1963), Beck & Ebert (1964). The oldest description of what we now call tympanosclerosis was published in 18 3 in Tröltsch's handbook *Ohrenheilkunde*. Here the disease was described as a sclerotic process in which tympanic mucosa becomes stiffer, more dense and less elastic. Furthermore Tröltsch reported that ankylosis occurs in the stapes and round window through calcareous deposits and bony changes. In connection with morphological findings of sclerosis in the mucous membrane of the tympanic cavity

Schwartze stated in 1878 in his handbook *Die Schleimhäute des Ohres und der Luftwege* that he found hyaline degeneration and dense collagenous fibers with mainly a little nucleus in the submucosal layer as well as tendigenous degeneration and the disappearance of vessels accompanied by fine calcareous granular depositions on the tympanic mucosa. In 1923 Brühl classified the sclerotic change which causes ankylosis in the stapes through a pathological change spread over all of the ossicular chain as chronic catarrhal otitis media. He emphatically differentiated these groups of diseases from otosclerosis.

With the use of antibiotics and the operating microscope there is frequent reconstructive surgery of the middle ear as well as stapes-surgery In the preoperative examination and during operating procedure there is ample opportunity to observe pathological processes taking place in the tympanic cavity In the findings made on tympanosclerosis made by Zöllner & Beck (1955) the histopathogenesis and diagnosis of tympanosclerosis as well as specific operative procedures were discussed in detail House & Sheehy (1960) brought out the fact that these pathological changes occur only in the submucosal layer of the tympanic mucosa, however Harris attempted to divide tympanosclerosis into two categories, i.e a benign "sclerosing mucositis and an invasive osteoclastic mucoperiostitis"

Although extensive light microscopic studies and clinical observations of the disease have been carried out, there are still unsolved problems relating the pathogenesis of this disease.

Hence the main purpose of this paper is an attempt to explain the origin and nature of the grotesque calcified masses in the middle ear space with help of the electron microscope and X ray microanalysis in the electron microscope

METHOD AND MATERIAL

For ultrastructural examination the sclerotic specimens were immersed immediately after the operative removal in cold (0–5 C) buffered 2.5% glutaraldehyde (pH 7.2 0 1 M phosphate buffer) cut into small pieces, kept in the refrigerator for 2 hours and washed overnight in cold buffer Postfixation was in cold (0–5 C) buffered 1% osmium tetroxide (pH 7.2 0 1 M phosphate buffer) for 90 minutes. All specimens were dehydrated in a graded ethanol series, passed through propylene oxide and finally were embedded in Epon according to Luft s method Thin sections were cut with glass knives on an L.K.B ultramicrotome. The sections were mounted on copper grids and were stained with uranyl acetate (10 min) followed by lead citrate (Reynold s method) (5 min) These sections were examined in a Carl Zeiss EM 9 electron microscope.

The other sections for light microscopy were cut at 0.5–1 0 μ and stained according to Kossa s method. The sections for X-ary microanalysis were cut at 0.3–0.5 μ in thickness and were mounted on gold grids without staining

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Fig 1 Electron-dense mitochondria nd conti nous collagen fibers from the membrane f fibrobl st-like cell. *M* Electron-dense mitochondria *MC*, membrane bound fragment with dense coat N nucleus. 22,400.

The collagen in transverse and longitudinal sections their electron density ranged in various grades. Some of them appeared to be indistinct, fusing and to anastomose each other (Figs. 2 and 3) At high magnification collagen fibers ran randomly superimposedly and interwavedly some of which contained membrane bound round fragments, so-called lysosome and mitochondria (Fig. 2)

Among the running collagen fibrils, fine dotted, numerous particles were seen These particles tended to aggregate and to have a circular arrangement (Figs. 2 and 3) In the circular aggregated region, fragmentated fibrils and electron-dense materials re present. Numerous continuous collagen fibers around the fibroblast like cell membrane were seen (Fig 4)



Fig 5 *Area f heavy calcificatio showing ggregati f apatite-like m terial t form* a coalescent mass. At the point of calcification, runni g collagen fibers ppear t be *disrupted. C, Coalescent calcificati n CF disrupted coll gen fibers. 1 Fine granular* material deposits I membrane-bound fragment 2, more dense gra ules with d nse point *were identified withi fragment; 3 doubl ring forming dense centers w re identified* 4 d uble rings with more dense centers were identified; 5, nnual-circl -like pl que f *calcification; 6, marked dense plaque with fusi g collagen fibers. 7 calcium plaques con-fluent with each other* 22,000.

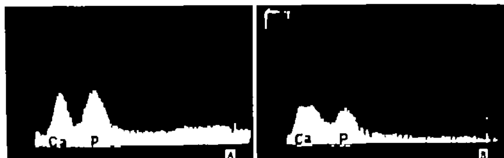


Fig. 6 ( ) X-ray spectrogram of the intracellular apatit Ca, Calcium wa P Phosphat w (*b*) X ra spectrogram f the patit i the collagenou bundles. C, Cal i m w P Phosphat w

Deposition of electron-dense material

Among the collagen, fibrils with electron-dense materials were randomly deposited in membrane-bound fragments. The membrane-bound fragments revealed a round or oval shape and measured about 0.2–1.5 μ in size (Fig. 2)

Some of these fragments appeared to contain finer granules, small dense centers rings forming dense centers were often identified These depositions of electron-dense materials forming lamellated arrangements tended to aggregate with each other These fragments formed in a doughnut-like shape in an annual ring-like form or tortoise-shell pattern (Fig. 5) At high magnification several parallel clefts were seen in each calcareous deposit plaque, and the electron-dense crystals were deposited on or close to the collagen fibrils rather than within them (Fig. 2)

At the site of the calcareous deposits, collagen bundles were revealed as suddenly disrupted, interwaved or superimposed Intracellularly marked electron-dense mitochondria, electron-dense degenerated so-called lysosomes and some of the fragments are seen (Fig 5)

The above mentioned electron-dense materials were proved as Ca^{2+} and phosphate crystals—probably apatite—through X ray micro-analysis. This X-ray diffraction showed that intracellular electron-dense materials con



Fig 7 X-ray spectrogram for rious substances. *I Ca*, larg intracell lar calci m plaque; *I P* large intracell la phosphat plaque *E P* large extracell lar calcareo plaque; *E Ca*, l rge calcareous plaque l collagenous fibers; *S Ca* small i tracell l calcareous plaque; *S P* small intracellular phosphat plaque.

tained more calcium than phosphate whereas extracellular electron-dense plaques contained more phosphate than calcium (Fig. 6) On the other hand, no significant lines were obtained by the following substances Cl, Fe Cu, Mg, Cr Mn, K S, Co, Zn, Sr etc. These facts suggested that the dense materials are calcium-phosphate crystals (Fig 7)

DISCUSSION

Normal middle ear lining mucosa consists of non-ciliated cuboidal epithelium on a thin tunica propria in the floor of the tympanum and then changes to a flat pavement epithelium in the epitympanum, mastoid antrum and pneumatic cells. Through chronically repeated and prolonged middle ear infection and invasion by fibroblasts, this thin tunica propria forms, with thick collagenous fibers. Furthermore, a hyaline degeneration is common to this type of tissue and if this takes place the fibers become indistinct, fusing into homogeneous mass. These changes may go on to calcification.

Hence under the ultrastructural relationship among cells, collagenous fibers and calcareous deposits are as follows The earliest recognizable change is shrinkage and increased density of the nuclear chromatin (pyknosis) The cytoplasm passes through variable degenerative changes which may include cloudy swelling by dystrophic, fatty or hyaline degeneration The cytoplasm fusing with the cell membrane to form an amorphous, granular opaque mass.

Various shaped electron-dense masses formed within the matrix of mitochondria of the fibrocyte-like cells (Figs. 1–3) This fact suggested early calcification within the mitochondria. The presence of fine granules or electron-dense materials in the marked dystrophied mitochondria, in the membrane bound fragment in the so-called autophaged lysosomes or in the collagenous fibrils indicates destruction of the fibrocyte-like cells and calcium deposits within mitochondria and in the so-called lysosomes (Figs. 2, 5 6)

Marked proliferated collagen fibers ran randomly or parallel in various directions in the extracellular space Within or close to collagen bundles, from the destroyed cells, various grade of the electron-dense materials were observed This suggested that degeneration of the collagen fibers with early calcium deposits takes place. At the site of the calcareous deposits arrangements of the collagen fibers became shortened, irregular disrupted and anastomosed each other Degenerating mitochondria among collagen bundles indicated its being scattered from ruptured fibrocyte like cell membrane into extracellular tissue (Fig 1)

Numerous collagen fibers with electron-dense materials close to or near the fibrocyte-like cell membrane were regarded as fibers produced in fibroblast after calcification had taken place These electron-dense masses in the collagen bundle within the degenerating mitochondria, so-called autophaged lysosomes in degenerating fibrocyte-like cells or among the collagen bundle

and in degenerating cell stroma among the collagen bundle proved to be calcium-phosphate crystals through X-ray micro-analysis, whereas Fe Cu, Mg, Cr Mn, K, S, Co, Zn, Sr Cl etc. were negative (Fig. 7)

The above-mentioned findings mean that this calcification of tympanosclerosis differentiates from normal bone calcification which is composed of CaPO (85–90% $CaCO_3$ (10–15%) MgSO (1.0–1.8%) and needle-like hydroxy apatite Furthermore it differentiates from lung tuberculosis which consists of lipoid-acid, calciumoxalate and calcium protein (Ratzenhofer & Propst 1953)

The pathogenesis of pathological calcification in various processes has been studied by biochemical and histochemical methods. In 1964 Cosmos and Lehninger pointed out that mitochondria play a key role in the physiological activity of calcium in the cell. The calcification of degenerated cells is not dependent on high concentration of calcium of the extracellular fluid From the foregoing it may be concluded that accumulation of calcium in mitochondria is independent of concentration of calcium, but the up-take of calcium by mitochondria is quantitative and in stiochiometric relationship between electron transport and ion accumulation.

In collagen bundles of the extracellular space the mitochondria changed probably by phosphorylation and the electron transport is changed in the degenerated cells. On the other hand the pH value dropped and calcium salts showed to have an affinity for the degenerate material in degenerating or necrotic tissue (Lehninger 1964)

Biochemically secondary impregnation of calcium in the extracellular space results probably from an elevated synthesis of mucopolysaccharide and chondroitinsulphate (Leughthenberger & Fautrez, 1960)

Probably latent hyperparathyroidism in a lower grade was present in the early stage of tympanosclerosis in childhood from which the early calcification results through a high level of calcium in the extracellular fluid. In an examination with guinea pigs we found that hyperparathyroidism is the cause of calcification of tympanosclerosis.

ZUSAMMENFASSUNG

Eine lichtmikroskopische Untersuchung zeigte Vermehrung von kollagenen Fasern hyaline Degeneration und dunkelblaue Verfärbung mit Haematoxilin in den Sklerosemassen. Durch elektro enmikrosk pische Untersuchung wurd n folgend Befunde an der oben erwähnten St ll festgestellt 1) Degeneration der M tochondrien mit lektronen-optisch-dichten Massen in fibrozytenähnlichen Zellen. 2) Elektronen-optisch-di htes Zellstroma, sog. Aut phagen Lysosomen und an M mbranen gebundene Fragmente in fibrozytenähnlichen Zellen oder zwischen kollagenen F serbündeln. 3) Di ht an der oder nahe der Zellmembran zahlreiche kollagene Fasern mit lektronen-optisch-dichtem Material. Diese T tsach wurde als neu produzierte Fibrillen in Fibroblasten ch der Calcifikation ufgefasst. 4) Durch Röntgen-Mikroanalyse im Elektronenmikroskop erwies sich dieses elektronen-optisch-dichte Material ls Calciumphosphatk istalle, wahrscheinl h

als Apatit Klümpchen. 5) Röntgenmikroskopische Analysen zeigten, dass intrazelluläres elektronen-optisch-dichtes Material mehr Calcium als Phosphat enthielt, während extrazelluläre elektronen-optisch-dichte Plaques mehr Phosphat als Calcium enthielten. Daraus kann geschlossen werden, dass es unsicher ist, ob in der extrazellulären Flüssigkeit eine Hypercalcinosis besteht. 6) Verschiedene andere Substanzen wie Fe Cl, Cu, Mg, Cr Mn, sind nur in geringen Mengen vorhanden. 7) Die Form der Apatit Klümpchen ähnelt einer doughnut in der Art von Jahresringen oder gemustert wie Schildpatt 8) Tierexperimente zeigten, dass Hyperparathyroidismus eine Calcification der Tympanosklerose verursachte

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PHONOPHOBIA AFTER STAPEDECTOMY

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Phonophobia is defined as an abnormal discomfort for suprathreshold sound which does not annoy healthy individuals. This study has demonstrated that phonophobia is present in all cases after stapedectomy and that the symptoms disappear within a year. The cause of phonophobia has been discussed, and lack of central nervous inhibition due to inactivity is found to be the most reasonable cause.

An abnormal discomfort or annoyance for suprathreshold sounds is a frequent or almost constant symptom in patients post stapedectomy as well as in many cases of facial palsy. The symptom has been given many names, such as hyperacusis, hyperacusis dolorosa, dysacusis. Tschiassny (1949) was the first who introduced the term phonophobia as regarding this symptom, in analogy with the term photophobia of the eye. He used the word "phonophobia" for designating the symptom of hypersensitivity for auditory stimuli. According to Jepsen (1955) phonophobia should presumably be regarded as part of the characteristic increased excitability in patients who feel discomfort and pain at sound intensities which do not annoy healthy individuals.

Phonophobia has been studied by Jepsen (1955) in cases of facial palsy. He found that this symptom was present only in cases with homolateral stapedius paralysis, and that phonophobia subsided before or simultaneously with the return of the stapedius function. He concluded that phonophobia in patients with peripheral facial palsy is due to paralysis of the stapedius muscle. However, he does not mention whether or not the symptom is disappearing in patients with persistent stapedius paralysis.

Tschiassny (1949) describes a case of stapedio-paralytic phonophobia in a deaf ear. In this connection he mentions that the symptom may also be met with in other cases, such as hemicrania, trigeminal neuralgia, cerebral affections, and especially in cases of severe otosclerosis. He suggests that the test for phonophobia may provide diagnostic support in cases of questionable otosclerosis.

Relatively few studies of the problem of phonophobia can be found, especially of the phenomenon in patients after stapedectomy for otosclerosis. Melnick (1968) in a study of loudness post-stapedectomy has discussed some of the problems of phonophobia. He could not demonstrate any differences in the loudness functions post-stapedectomy as compared with nor

mal listeners. He concluded that the patients in his experiments might have been seen after a process of adaptation might have reduced any changes in loudness resulting from cutting of the stapedial tendon (2–21 months after surgery)

MATERIAL AND METHOD

Seventeen patients with clinical otosclerosis were tested for phonophobia *after stapedectomy In all cases there has been used a polystan-strut* technique with fascia from the temporal muscle covering the oval window The stapedius tendon was cut in all cases. These patients are routinely controlled 1 3, 6 12 and 24 months after surgery and the tests have been performed at these intervals The patients were examined for symptoms of phonophobia and tested with an Amplivox audiometer calibrated after a modified ISO standard [1] and a maximum output of 110 dB

The test was performed in the same way as by Jepsen (1955) recording the threshold of phonophobia for pure tones in connection with the determination of the threshold of hearing Interrupted tones of increasing intensities *were presented monaurally to the affected ear the patient being* instructed to give a sign when the sound became uncomfortable or painful. The following frequencies were used 125–250–500–1000–2000–3000–4000–6000–8000 Hz.

RESULT

Ten normal listeners with hearing threshold 0–10 dB were tested for phonophobia None of them complained of phonophobia of the same magnitude as the stapedectomized, but some of them reported a mild discomfort at the maximum output of the audiometer in the range 500–2000 Hz, i.e at a *level of 100–110 dB above hearing threshold.*

Table 1 gives an indication of the intensities required for eliciting phonophobia in the different frequencies. The intensities above zero level (2×10^{-4} N/m²) as well as above hearing level are quoted It must be emphasized that there is very little spread in the values above hearing threshold eliciting phonophobia in the different frequencies from case to case. In the cases where phonophobia has been found 1 3 and 6 months after stapedectomy it has been elicited at the same intensities above hearing *threshold* each time

In nearly all cases phonophobia had been noticed by the patients in advance. Many of them described extreme annoyance or even pain when exposed to noises of different kinds, such as traffic noise, loud speech, noise from doors and so on The symptom was usually noticed immediately after surgery and apparently independent of the postoperative hearing thresh

[1] Equivalent threshold levels in dB re 2 10^{-4} N/m 125 Hz, 45 0· 250 Hz, 25.5, 500 Hz, 11.6, 1000 Hz, 7 0· 1500 Hz, 6.5, 2000 Hz, 9 0· 3000 Hz, 10.0· 4000 Hz, 9 6, 6000 Hz, 15.5, 8000 Hz, 13.0.

Table 1 *Mean thresholds for phonophobia*

Hz	125	250	500	1000	2000	3000	4000	6000
Threshold above zero level, dB	55	75	105	100	105	105	105	75
Threshold abo hearing level, dB	40	70	75	75	75	65	55	45

Table 2 *Sensation of phonophobia (duration after stapedectomy)*

Duration	No. of cases
N phonophobia	4
1 7 days	1
1–4 weeks	6
2–3 months	5
4–6 months	1

Table 3 *Hearing threshold in the patients after stapedectomy*

dB	No. of cases
1 10	2
11 20	9
21 30	3
31–40	2
41 50	1

old, which could not be demonstrated elevated at that time (Table 2) The sensation of phonophobia lasted for 1–3 months in nearly all cases (Table 2)

At the time of investigation, the threshold of hearing was within the limits of social hearing in most cases, i.e hearing level ≤30 dB in the range 500–1000–2000 Hz (Table 3) In most cases there was complete closure of air–bone gap

As a result of audiometric examination for phonophobia to pure tones, the following could be stated (in addition to what has been referred to in Table 2) Phonophobia was present in all patients one month after stapedec tomy Three months after surgery phonophobia could be demonstrated in about one-half of the cases. Six months postoperatively about 30% still had phonophobia. One year after stapedectomy or later phonophobia was absent in absolutely all cases.

Table 4 shows the distribution of the frequencies at which the phonophobia could be elicited. Most frequently the phenomenon is present in the range from 500 to 4000 Hz, with a maximum at 2000 Hz, but in a few cases it could be elicited in the whole range from 125 to 6000 Hz. For 8000 Hz, phonophobia has not been present in these patients. This may depend on the high-tone loss present in many of the cases.

DISCUSSION

In 17 patients with otosclerosis, phonophobia could be demonstrated one month after stapedectomy It has also been found that phonophobia disappears in most cases 3–6 months postoperatively and in all cases within one year after surgery

Table 4 *Phonophobia elicited at different frequences one month after stapedectomy*

Case no. Hz.	125	250	500	1000	2000	3000	4000	6000	8000
1					1	1			
2			1	1	1	1	1		
3					1	1	1		
4			1						
5			1						
6			1	1	1	1	1	1	
			1	1	1	1	1		
8			1	1	1	1	1		
9				1	1		1		
10	1	1	1	1	1		1	1	
11			1	1	1				
12					1	1			
13					1				
14		1	1	1	1	1	1		
15			1	1	1	1			
16				1					
17		1	1	1	1	1	1		
Total	1	3	11	11	14	10	9	2	

Phonophobia is most frequently elicited from 500 to 4000 Hz, and at a level of 7o dB above hearing threshold in the range 500–2000 Hz.

Phonophobia may be due to one or more of the following mechanisms

1 Lack of protection of the inner ear from the contraction of the stapedius muscle.
2. A change in the sound transmission of the middle ear possible because of a change in the mass or/and a modified connection between the different parts of the sound transmission system.
3 Cochlear reaction (labyrinthitis?)
4 Change in the central nervous inhibition due to inactivity for years.

COMMENT

1 Stapedio-paralytic phonophobia in Bells palsy is a well known phenomenon and Jepsen (1955) found that phonophobia disappeared when the reflex contractions of the stapedius muscle returned In post stapedectomy patients the disappearance of phonophobia cannot be explained in this way since the stapedius tendon has been cut.

2. It is obvious that changes in mass due to the prosthesis takes place and also that the connection between incus /prosthesis/ oval window in relation to the normal stapes is altered This may lead to a change in amplitudes,

with discomfort or pain as a result It is difficult to explain the subsiding of phonophobia post-stapedectomy due to the mechanisms mentioned in 1 and 2. Different prostheses or techniques do not alter phonophobia. The symptom was also present—subjectively at least—in the previous mobilization operations. (This has naturally not been possible to prove by objective registrations.) There is, however *a possibility that adaptation takes place* postoperatively

3 In the report of Melnick (1968) studies based on monaural equal loud ness *and magnitude estimation experiments did not reveal any change in* loudness function post stapedectomy According to these results, phonophobia should not be due to a cochlear reaction.

4 This study has not solved the problem of phonophobia or the cause of *the phenomenon. It is,* however reasonable to promote the following hypothesis Due to inactivity for a long time most often for years, the central nervous inhibition on the auditory impulses has been put out of function, and it will therefore take some time to activate this mechanism.

ZUSAMMENFASSUNG

Ph ophobie kann als ein abnormes unangenehmes Gefühl gegenüber höheren Tönen, das nicht bei gesunden Menschen auftritt, bezeich et werden. Siebzehn Patienten si d nach St pedectomie auf dieses Sympt m untersucht worden In sämtlichen Fällen war nach einem M nat post operationem dieses Sympt m eindeutig vorha den Bei sämtlichen Patienten war Phon phobie nach 1 em Jahr nicht meh nachweisbar Unterschiedliche Ursachen der Phonophobie werden erörtert.

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BIOCHEMICAL STUDIES OF OTOSCLEROSIS

Protein and Enzymes in Stapedes and Cortical Bone

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Significant differences in the amounts of extracted protein and in lactate dehydrogenase activity were found between normal cortical bone obtained from the inner end of the posterior metal wall and cortical bone from the same area in otosclerotic patients. The amount of protein extracted was 32% lower in cortical bone from otosclerotic patients. The activity of lactate dehydrogenase was 51% lower on a per milliliter extract basis and 6% lower on a per microgram protein basis in cortical bone obtained from otosclerotic patients as compared to normal cortical bone. Since cortical bone is rarely affected by otosclerosis, these results could support the hypothesis, previously suggested, that the disease is a manifestation of a generalized metabolic disorder characterized by biochemical changes in the supporting tissues.

Differences were also found in the biochemical composition of stapedes from non-otosclerotic individuals as compared to stapedes from otosclerotic patients. The activity of lactate dehydrogenase was lower in otosclerotic stapedes on both a per milliliter extract basis (percentage decrease 38%) and a per microgram protein basis (percentage decrease 49%). The activity of malate dehydrogenase was also lower in otosclerotic stapedes though not to the same degree as lactate dehydrogenase. An eightfold increase in alkaline phosphatase activity was found in otosclerotic stapedes as compared with normal.

These results suggest that otosclerosis is characterized by abnormal levels of enzyme activity, notably by decreases in lactate and malate dehydrogenase activities and by an increase in alkaline phosphatase activity.

Differences in biochemical composition have been reported between normal and otosclerotic mallei and incudes (Soifer *et al.*, 1967). Fifty per cent more protein was extracted from mallei and incudes obtained from patients with clinical otosclerosis than from mallei and incudes from non-otosclerotic individuals. Differences in the activities of various enzymes were also found to occur between the normal outer ossicles and those obtained from otosclerotic patients. These observations suggest that otosclerosis is a manifestation of a generalized metabolic disorder characterized by biochemical changes in the supporting tissues (Soifer *et al.* 1965, Arslan & Ricci 1960, Ricci, 1962). To verify this hypothesis, the study of protein and enzyme

activity reported in this paper was carried out on samples of cortical bone obtained from otosclerotic and non-otosclerotic individuals.

The authors have also shown that normal stapedes have a higher metabolic activity when compared with normal mallei and incudes (Solfer *et al.*, 1967) This is indicated by increases in extracted protein and in the activities of certain enzymes such as lactate dehydrogenase malate dehydrogenase aspartate aminotransferase and aldolase The logical extension of the information to date is an investigation to determine whether stapedes with otosclerotic foci have a higher metabolic activity than those which are unaffected by the disease Such a study is reported in this paper

MATERIAL AND METHODS

Stapedes and samples of cortical bone from the inner end of the posterior meatal wall were obtained from patients with clinically-diagnosed otosclerosis, confirmed at surgery Stapedes removed at autopsy from non-otosclerotic patients were used as controls. Control specimens of cortical bone were obtained from patients undergoing tympanoplasty and having no clinical evidence of otosclerosis. Since the surgical samples were unavoidably contaminated with blood, it was necessary to rinse them in a small quantity of Ringer s-lactate solution. The specimens were then blotted well to prevent extraction during storage by any remaining rinse solution, wrapped in foil, and frozen.

The stapedes and samples of cortical bone were extracted and assayed for total protein and for the activities of lactate dehydrogenase malate dehydrogenase glucose-6-phosphate dehydrogenase aspartate aminotransferase aldolase acid phosphatase and alkaline phosphatase according to methods used previously (Solfer *et al* 1967)

Extracted protein is expressed as micrograms of protein per milligram wet weight of bone Alkaline phosphatase activity is expressed as micromoles of substrate converted per minute per milligram wet weight of bone The activities of the other enzymes are expressed in two ways (I) as micromoles of substrate converted per minute per milliliter of extract and (II) as micromoles of substrate converted per minute per microgram of extracted protein.

RESULTS

The results of the determinations of extracted protein and of various enzyme activities in cortical bone from otosclerotic and normal (non-otosclerotic) patients appear in Table 1 The average value of extracted protein in cortical bone from otosclerotic patients represents a percentage decrease of 32 when compared to that of cortical bone from non-otosclerotic patients. The activity of lactate d hydrogenase i lower in cortical bone from otosclerotics than in normal bone both on a per milliliter extract basis (per

Table 1 *Protein and enzymes in cortical bone*

Tissue	Extracted protein[a]	LDH I	LDH II	MDH I	MDH II	AAT I	AAT II	Aldolase I	Aldolase II	G-6-P I	G-6-P II	Acid phosphatase I	Acid phosphatase II	Alkaline phosphatase[b]
Otosclerotic cortical bone														
Pool[c]														
1	13.2	135	10	24	2	38	3	31	2	None detected		3.8	0.3	0.9
2	12.6	155	12	24	2	45	4	25	2	None detected		4 4	0.3	0.9
3	11.2	164	15	20	1	34	3	48	4	Non detected		6.8	0.6	1.8
4	9 0	146	16	14	2	25	3	22	4	None detected		3.0	0.3	0.7
5	13.0	164	13	14	1	34	3	27	2	N ne detected		3.8	0.3	0.4
6	14.3	232	16	19	1	38	3	27	2	1.3	0.06	3.4	0.2	0.7
7	16.3	152	15	24	2	40	3	31	2	0.7	0.04	3.7	0.2	0.9
8	17.0	194	11	24	1	33	2	21	1	1.0	0.06	3.8	0.2	0.9
Av	13.3	180	14	20	2	36	3	30	2	0.4	0.02	4 1	0.3	0.9
Normal cortical bone														
Pool														
1	19.5	406	21	34	2	51	3	33	2	2.4	0.12	9.3	0.5	1.5
2	19.8	329	17	29	2	46	2	26	1	1.5	0.08	4 6	0.2	1.0
Av	19.7	368	19	32	2	49	3	30	2	2.0	0.10	7.0	0.4	1 6

[a] E pressed micrograms f prot in extracted per milligram wet weight of bone

[b] Expressed 10[illegible] micromoles f substrat converted per min t per milligram wet weight of bone.

[c] *Contains bone tiss from 20 t 30 patients.*

I expressed a 10[illegible] micromole of substrat co verted per mi ut per milliliti of extract

II Expressed a 10[illegible] micromoles of substrate con erted per min t per microgram of protein.

centage decrease 51%) and on a per microgram protein basis (percentage decrease, 26%) This indicates that the decrease in lactate dehydrogenase activity is less than that of total protein and represents a statistical difference between the normal and otosclerotic tissues. The activities of malate dehydrogenase aspartate aminotransferase, and glucose-6-phosphate dehydrogenase are lower in cortical bone from otosclerotic patients on a per milliliter extract basis, but they are the same for otosclerotic and normal bone on a per microgram protein basis. Therefore, the apparent low values for otosclerotic tissues merely reflect the decrease in extracted protein. No differences between normal and otosclerotic cortical bone are found for aldolase or for acid phosphatase The activity of alkaline phosphatase appears to be lower in cortical bone from otosclerotic individuals. However due to the overlapping of the values for the two types of bone and the relatively small number of normal values, this difference is probably only apparent

The results of the protein and enzyme study carried out on stapedes from

Table 2. *Protein and enzymes in stapedes*

Tissue	Extracted protein	LDH I	LDH II	MDH I	MDH II	AAT I	AAT II	Aldolase I	Aldolase II	G-6-P I	G-6-P II	Acid phosphatase I	Acid phosphatase II	Alkaline phosphatase[b]
Otosclerotic stapedes														
Pool														
1	18.3	920	60	56	3	97	5	72	4	4.8	0.3	8.2	0.5	13.2
2	24.4	832	31	56	2	81	4	66	3	4.8	0.2	7.0	0.3	9.6
3	25.0	1026	41	73	3	113	5	83	3	6.6	0.2	9.8	0.4	14.8
4	23.8	1374	48	82	3	104	4	89	3	5.6	0.2	9.8	0.3	15.4
5	32.5	1277	39	82	3	100	3	89	3	8.0	0.3	11.4	0.4	23.7
6	27.4	1045	38	87	3	63	3	115	4	7.3	0.3	None detected		—
7	25.0	881	35	77	3	55	2	85	3	7.3	0.3	None detected		—
8	22.0	871	35	77	3	53	2	81	4	—	—	None detected		—
9	23.8	871	37	58	3	51	2	60	3	5.7	0.2	—	—	6.4
10	17.0	689	37	73	4	97	6	55	3	4.4	0.3	8.4	0.4	3.3
11	20.0	745	37	82	4	106	5	56	3	4.6	0.2	7.4	0.4	4.7
Av	24.8	952	39	73	3	85	4	77	3	5.8	0.3	8.5	0.3	11.4
Normal stapedes														
Pool														
1	19.3	1384	72	88	5	116	6	91	5	5.8	0.3	10.2	0.5	1.5
2	22.9	1529	67	87	4	109	5	100	5	7.6	0.3	7.6	0.3	1.6
3	18.8	1665	89	97	5	90	5	92	5	6.3	0.4	6.7	0.4	1.2
Av	20.3	1526	76	91	5	106	5	94	5	6.6	0.3	9.2	0.4	1.4

Expressed as micrograms of protein extracted per milligram wet weight of bone.

Expressed as 10^{-4} micromoles of substrate converted per minute per milligram wet weight of bone.

Contains bone tissue from 20 to 30 patients.

Quantity insufficient for determination.

I. Expressed as 10^{-4} micromoles of substrate converted per minute per milliliter of extract.

II. Expressed as 10^{-4} micromoles of substrate converted per minute per microgram of protein.

otosclerotic and non-otosclerotic patients are shown in Table 2. No differences are observed between normal and otosclerotic stapedes either in the amount of extracted protein or in the activities of aspartate aminotransferase aldolase, glucose-6-phosphate dehydrogenase or acid phosphatase. The activity of lactate dehydrogenase is lower in otosclerotic stapedes on both a per milliliter extract basis (percentage decrease, 38%) and a per microgram protein basis (percentage decrease 49%). The activity of malate dehydrogenase is also decreased in otosclerotic stapedes though not to the same degree as lactate dehydrogenase the percentage decrease of malate dehydrogenase in otosclerotic as compared to normal stapedes is 20% on a per milliliter extract basis and 40% on a per microgram protein basis. The

indication that stapedes having otosclerotic foci are lower in lactate and malate dehydrogenase activity than stapedes which are unaffected by the disease process is particularly interesting since the protein content is the same in diseased and normal tissue The increase in alkaline phosphatase activity occurring in the involved stapedes is striking The average activity in otosclerotic stapedes is approximately eight times that of normal stapedes.

COMMENTS

The results of the cortical bone studies are different from the results of previous studies carried out on other tissues normally unaffected by otosclerosis (Soifer *et al* 1967) For example values for extracted protein and for lactate dehydrogenase activity are lower in cortical bone obtained from individuals with otosclerosis than in normal cortical bone while protein and lactate dehydrogenase activity are higher in mallei and incudes from otosclerotic patients than in ossicles from non-otosclerotic patients. However the fact that differences are observed between otosclerotic and non-otosclerotic individuals supports the hypothesis previously suggested that otosclerosis is a generalized metabolic disorder characterized by biochemical changes in the supporting tissues. The fact that the differences may or may not be in the same direction probably reflects the variations inherent in the tissues themselves.

Histochemical studies have shown that otosclerotic foci are metabolically active particularly during the stage of growth and invasion From results of qualitative histochemical studies of several enzymes in two otosclerotic footplates, Ardouin & Wegmann (1961) have suggested that the changes occurring as a result of the disease process may be quantitative and may actually represent a decrease rather than an increase in enzyme conent. Chevance *et al* (1962) concluded from histochemical studies of otosclerotic footplates that no single enzyme change is responsible for producing the otosclerotic lesion but rather that many are involved each at a different focal stage Alberti & Tarkannen (1963) also suggest that no one enzyme disorder exists in clinical otosclerosis but rather that an upset in enzyme balance characterizes the disease

The results of the biochemical study of otosclerotic and normal stapedes reported in this paper show that lactate and malate dehydrogenase activity are decreased the activities of aspartate aminotransferase, aldolase glucose-6-phosphate dehydrogenase, and acid phosphatase are the same and alkaline phosphatase activity is greatly increased as a result of otosclerosis. Though correlation between histological and biochemical studies is necessary to fully elucidate the etiology of the disease, this must await a more extensive biochemical characterization of the lesion. However the results of the present study do indicate a direction for further investigations. The results also suggest that otosclerosis is characterized by abnormal levels of

enzyme activity notably by decreases in lactate and malate dehydrogenase activities and by an increase in alkaline phosphatase activity

Of particular interest is the eightfold increase in alkaline phosphatase activity in otosclerotic stapedes as compared with normal stapedes. This is in direct conflict with the observation of Maurer (1961/62) of decreased alkaline phosphatase activity in otosclerotic stapedes. The precise role played by alkaline phosphatase in the calcification of skeletal tissues is still unknown. In addition to its connection with glycolysis, alkaline phosphatase may be implicated in the formation of the organic bone matrix itself i.e., in the production of collagen fibrils. Neuman (1956) theorized that units are synthesized inside the cell as phosphate esters. These esters are then secreted at the surface of the cell where they are dephosphorylated by the extracellular phosphatase and combine to form fibrils. This would explain the presence of extracellular phosphatase in the newly forming matrix prior to the actual deposition of bone salt as well as the presence of the enzyme at sites of fibrinogenesis. Alternatively alkaline phosphatase may play a role in the synthesis of the matrix in the form of mucopolysaccharides (Weidmann, 1963) Kroon (1952) suggested that alkaline phosphatase may provide the units essential for the synthesis of mucopolysaccharides by liberating them from hexose phosphate esters produced during glycolytic breakdown Studies are being carried out by the authors on the mucopolysaccharide (hexosamine) and collagen (hydroxyproline and proline) content of ossicles and of various regions of the temporal bone (Soifer *et al* 1968, in preparation) It is hoped that a correlation of the results of these studies with alkaline phosphatase activity may elucidate the role played by the enzyme in otosclerosis.

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ZUSAMMENFASSUNG

Bedeutsame Unterschiede in der Menge des extrahierbaren Proteins und in der Laktat Dehydrogenase-Aktivität konnten im kortikalen Knochen des inneren Endes der hinteren knochernen Gehörgangswand von normalen Individuen und von Otosklerotikern nachgewiesen werden. Die Menge des extrahierbaren Protei im kortikalen Knochen war bei Otosklerotikern 32% weniger als bei Nicht Otosklerotikern. Die Laktat-Dehydrogenase-Aktivität der Otosklerotiker war 51% weniger auf der per Milliliter Extrakt Basis und um 20% weniger auf der per Microgram Protein-Basis, als normalerweise. Da der kortikale Knochen praktisch niemals von Otosklerose befallen ist, konnten diese Befunde für die schon früher in Erwägung gezogene Hypothese sprechen, dass die Otosklerose eine Manifesta-

tion einer allergemeinen Stoffwechselstörung ist die durch biochemische Ver änderungen in den Stützsubstanzen gekennzeichnet ist

Weiter wurden Unterschiede in der biochemischen Zusammensetzung der Steigbügel von normalen und von Otosklerotikern nachgewiesen Die Laktat Dehydrogenase-Aktivität in Otosklerotiker Steigbügeln war weniger sowohl auf der per Milliliter Extrakt Basis (38%) als auch auf der per Microgram Protein-Basis (49%) Die Malat Dehydrogenase-Aktivität in Otosklerotiker Steigbügeln war ebenfalls weniger wenngleich nicht in demselben Ausmasse Weiter wurde eine *achtfache Erhöhung der alkalischen Phosphatase-Aktivität in otosklerotischen* Steigbügeln im Vergleich zu normalen Steigbügeln festgestellt. Diese Ergebnisse sprechen dafür dass die Otosklerose durch eine abnorme Aktivität gewisser Enzymen in erster Linie einer verminderten Laktat und Malat Dehydrogenase und einer sehr starken Erhöhung der alkalischen Phosphatase-Aktivität gekennzeichnet ist.

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VIII NERVE IN EXPERIMENTAL LEAD POISONING

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Forty healthy guinea pigs were poisoned with repeated peritoneal injec tions of lead cetat After 7 weeks the animals were sacrificed and the I ner ear with VIII nerve were examined histologically The lead level in th blood was estimated in all animals fter poisoni g and in 10 healthy guinea pigs (control group) The sensory cells of the i ner ear the spiral and vestibular ganglion cells ppeared t be normal. Th VIII nerve in the majority of poisoned animals showed segmental dem eline-tion and axonal degeneration Theories on etiology and pathogenesis f th neurop th logical changes in lead poisoning are discussed.

Among the many symptoms of lead poisoning are found complaints of auditory defects and vertigo (Ciurlo & Ottoboni, 1956 Falkowska *et al.*, 1964 Gammarroto & Bartoli 1964 Ursan & Suciu, 1965) The answer to the problem of the toxic effect of the lead on inner ear and the VIII nerve in man is still under discussion. Gambault (1880) was the first who demon strated peripheral nerve changes in guinea pigs resulting from lead poison ing. The next experiment reports described the influence of lead upon the central nervous system and peripheral motor nerves (Ferraro & Hernandez, 1932 Fullerton, 1966 Kornofsky & Ridgway 1952 and others)

The present work was undertaken to study the effect on the inner ear and VIII nerve after lead poisoning

METHOD

Fifty healthy young guinea pigs were used for the experiment Their weights ranged between 300 and 350 g Ten guinea pigs served as a control for the estimation of normal lead levels in the blood Forty animals were given repeated doses of 1% solution of lead acetate Pb/NO / by intraperi-toneal injections. The total dose of 300 mg/kg was administered one time every 7 days during 7 weeks. Eight animals died before the end of the experi-ment All remaining guinea pigs were weak and had lost weight During the poisoning their growth was inhibited. The loss of weight is, according to Fullerton (1966) and Causey (1965) a significant sign of serious poisoning Only five animals were able to run slowly but they appeared to have mild paralysis of their hind limbs After seven weeks the animals were sacrificed, the temporal bones removed for histopathological examination and the lead level in the blood was estimated. The latter was determined in all animals



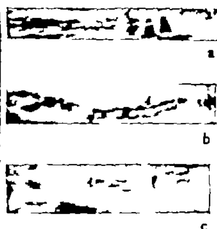


Fig 1 *Fig 2.*

Fig 1 The VIII nerv from the poisoned guinea pig (cross secti n) stai ed by Suda bla k B to show d my linati n. Th bundles of demyelinated fibres are present in the center f the trunk × 100. At the right f the figure the vestibula ganglion cells are seen.

Fig 2 VIII nerv —singl fibre from poisoned animal stained by H idenhain method (*a*, *b*) and Sudan black B ( ) (1) Segmental demyelination with the ovoid f deg nerat ing myelin. (2) Intact myelin sheath. Magnificati n ( ) × 1600, (b) 2600, (c) × 3200

after poisoning and in 10 healthy guinea pigs (control group) The estimation of lead was made by a polarographic method similar to that described by Teisinger *et al.*, (1956) The lead level in control group was 0 025 mg% – 0 33 mg% This level in the poisoned animals increased about 10 times to 0.31 mg% – 0 42 mg% For histopathological examinations the animals were divided into two groups. The temporal bones of 16 guinea pigs (first group) after fixation and decalcification in EDTA were embedded in celloidin, and sections were cut at 15–20 μ and stained with haematoxylin-eosin, cresyl violet and by silver impregnation method described by Gomori.

The temporal bones from the remaining 16 guinea pigs (second group) were embedded in paraffin, because the application of methods to demonstrate myelin and axon are limited by celloidin technic. Paraffin sections were stained by modified Heidenhain s method, Sudan black B and luxol fast blue for myelin sheaths and Roger Foot s method to demonstrate axons.

RESULTS

The histological investigations sho pathological in the sensory cells of the inner ear or in vestibular g . In



Fig 3 VIII nerve from normal guinea pig stained by Roger-Foot method to demonstrate axons. × 60.

the first group (celloidin embedding) VIII nerves from 12 of the 16 poisoned animals were histologically abnormal Focal changes with destruction of the nerve were seen in specimens stained by hematoxylin–eosin and by the silver method. There was individual variation in the degree of these changes. Detailed information about the character of these pathological changes was obtained by histopathological examination with staining by methods demonstrating myelin sheaths and axons. The VIII nerve of the 5 lead-poisoned animals in the second group was normal (Fig. 3) The VIII nerve of the remaining 11 guinea pigs *showed both demyelination and axonal degeneration*

On the cross section of the VIII nerve we observed characteristic changes when the nerve was stained by Sudan black B (Fig. 1) The demyelination spread to involve the nerve fibers in the centre of the trunk. Only residual fragments of myelin sheaths can be seen here The various stages of segmental demyelination in a single fibre were observed also in remaning parts of the nerve On the longitudinal sections (Fig 2) several ovoids of degenerating myelin can be seen.

Process of axonal degeneration in the same nerve is shown in Fig 4 The degree and extent of these changes varied in different parts of the same nerve. This distribution of the lesion prompts the suggestion that *in lead poisoned guinea pigs segmental demyelination and axonal degeneration of the VIII nerve occur*

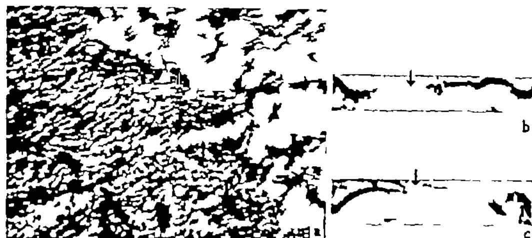


Fig 4 A nal degen ration in VIII nerv from poisoned animal Roger Foot stain. ( ) At th right f the figure l the regi n f total destruction f the nerv 400 (*b c*) A l *d ge rati l ingl fibre at higher mag ification. 3200.*

DISCUSSION

A full understanding of the pathogenesis of neuropathological lesions produced by lead has not emerged. The theory which was supported for many years is that of porphyrin metabolic deficiencies. Some authors have stressed damage to the vascular epithelium, suggesting that the neuropathological lesions may be secondary to the vascular (Cumings, 1959 Ursan & Suciu 1965) The predominant changes in the central nervous system were found by other workers

In previous experimental works on chronic lead poisoning demyelination of the nerve fibers was found (Fullerton, 1966 Glees, 1961) This toxic effect is probably the result of disturbance to the enzymatic content of myelin sheath. The degeneration of myelin may also be the result of the degene ration of the axis cylinder These biochemical lesions produced by heavy metals were stated by Glees (1961)

The striking resemblance of the early neurological symptoms of chronic lead poisoning in man to those seen in other demyelinating syndromes suggest that this is the same etiological agent For this reason some authors have suspected lead as a possible cause of multiple sclerosis (Campell, 19 0 Cone *et al* 1934 Falkowska *et al.*, 1964)

Our work showed that lead poisoning in guinea pigs might produce chronic, demyelinating neuropathy of the VIII nerve with axon degeneration. The sensory cells of the inner ear have been found to be most resistant to the toxic effect of lead. The degree of the histopathological changes is not necessarily the same in different animals. This finding suggests the individual sensitivity to lead intoxication and confirm other experimental and clinical observations.

The results of our experiment in guinea pigs are relevant with reference to lead intoxication in man, but may suggest the pathogenesis of VIII nerve lesions.

ZUSAMMENFASSUNG

Die Untersuchungen wurden an 40 gesunden jungen, weissen Meerschweinchen durchgeführt. Die Meerschweinchen wurden mittels intraperitonealer I jektionen von Bleiazetat allmähli h vergiftet. Nach einer 7 Wochen lang andauernden Ver giftung wurden die Tiere getötet und das Innenohr sowi der VIII Hirnnerv hist logisch u tersucht. Bei allen ergifteten Tieren und bei 10 gesunden Meer schweinchen, die als Kontrollgruppe dienten wurde de Bleig halt im Blut bestimmt. Die Sinnesz llen des Innenohres und die Nervenzellen des vestibulären Ganglions sowie des Ganglio spirale cochleae waren n rmal. Bei der M hrzahl der vergifteten Tiere wurd im VIII Hirnnerv eine segmentäre Demyelinisatio und eine Ax degeneration festgestellt. In de vorliegenden Arbeit wurden gleich f lls die besteh nden Auffassu gen uber die Ätiopathogenese der neuropatholo gi hen Veränderungen bei Vergiftungen dargelegt.

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COCHLEAR DAMAGE FROM OTOTOXIC ANTIBIOTICS BY INTRATYMPANIC APPLICATION

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Cochlear damage resulting from intratympanic application of neomycin, polymyxin B and colimycin was studied experimentally in guinea pigs. The cochleas were examined histologically according to the method of Engström and sensory cell loss was recorded graphically. It was found that relatively low concentrations of the drugs caused damage to the organ of Corti. On the basis of the findings, the possible diffusion routes of the antibiotics in the fluid spaces of the inner ear and the dangers arising from their topical use are discussed.

The ototoxicity of Streptomyces-antibiotics in systemic medication has been known for more than twenty years, and is well documented both clinically and experimentally. Fortunately, owing to the common knowledge of this fact, new cases of deafness from these drugs are rare today. Much less attention, however, has been paid to the ototoxicity of these antibiotics when applied locally for chronic otitis media. Both experimental and clinical data confirmed this risk in the case of streptomycin (Schuknecht, 1957; Spoendlin, 1966), but in at least one experiment it could not be established with neomycin (Riskaer *et al.*, 1956).

Three antibiotics were studied in the present experiment. Neomycin has long been the antibacterial agent in several brands of ear drops. Two antibiotics not belonging to the Streptomyces-group, polymyxin B and colimycin, are also used for the same purpose—often in combination with neomycin—because of their activity against pseudomonas.

MATERIAL AND METHODS

The three drugs were studied in the form of solutions, their lowest concentrations corresponding approximately to those in commercially available ear drops. Thus neomycin (Mycifradin sulphate, Upjohn) was applied in concentrations of 5, 10, 20, 50 and 100 mg/ml, polymyxin B (Polymyxin B sulphate, Pfizer) in concentrations of 1, 2, 5, 10 and 25 mg/ml and colimycin (Colimycin, Orion) in concentrations of 2, 5 and 10 mg/ml. The experimental animals, young guinea pigs with positive pinna reflex, weighing on an average 250 g, were anesthetized with intraperitoneal pentobarbital sodium. Using an operating microscope the tympanic membrane was visualized and punctured with a thin cannula and the middle ear filled with the solution

under study carefully avoiding other damage to the membrane and manipulation of the ossicles.

In this way 14 guinea pig ears were treated with neomycin, 15 ears with polymyxin B and 20 ears with colimycin. The control material consisted of 17 ears injected with physiologic saline solution or—to study substances having larger molecular weight—with solutions of glucose or sodium G penicillin in concentrations of the same osmotic pressure as the antibiotic solutions studied. The animals were killed by decapitation, the temporal bones removed, and the cochleas fixed immediately by perfusion with cold veronal buffered 1.5% osmium tetroxide solution The cochleas were dissected and a sample of about 1/ turn removed from each of the four coils of the organ of Corti. These samples were studied under phase contrast microscope the cellular damage assessed and registered in cochleograms according to the method of Engström (Engström *et al* 1966)

Another technique for topical application of neomycin was used in a group of 14 guinea pigs. In these animals the pars tensa of both tympanic membranes was removed, avoiding manipulation of the ossicles. The right ears of the animals were treated with neomycin in concentrations of 5 10 20 50 and 100 mg/ml by filling the external canal with the solution. The treatment was continued for 8 to 25 days once daily the animals were then killed and studied histologically as described. The left ears of the animals were kept for control purposes.

RESULTS

All control cochleas were histologically normal. The group of ears treated with antibiotics also included several which were undamaged, but in the majority there were degenerative changes ranging from just discernible alterations in a few sensory cells to complete destruction of the organ of Corti

The slightest discernible sign of beginning degeneration in the organ of Corti is a peculiar disarrangement of the sensory hairs on the outer hair cells, distorting the normally orderly W-pattern of the hairs (Fig. 1) This phenomenon has been described earlier (Hawkins & Engström, 1964) and probably is a result of abnormal movement of the hair rootlets in the pathologically softened cuticula of the hair cell With advancing damage sensory cells degenerate or "collapse" and so are easily identifiable among the remaining cells (Fig 2) Usually at least part of the inner hair cells remain undamaged at a stage of degeneration when all outer hair cells have been destroyed and even some of the Deiters cells damaged (Fig. 3) The advanced stage of degeneration, when all sensory cells have been lost, is usually associated with destruction of the supporting framework of the organ of Corti (Fig 4)

The loss of hair cells in the samples of each turn of the organ of Corti was mapped in cochleograms, where the degree of damage could be assessed and



Fig 1 Guinea pig P 10 S. Surf ce preparation 2 / coils from base of cochlea. 51 gl intratympanic i jection f polymyxi B 2 mg/ml On the first (1) row f uter hair cell the W formed by the sensory hairs (*H*) ppears dist rted. The hairs of the i er hair cell (*IHC*) are visible above th ribbon f the pilla heads (*P*) Phase contrast 650.



Fig 2 G inea p g N 13 D Surface preparatio I coil from base of cochlea. *After* removal f the pars tensa f the tympa ic membrane the external canal ha been filled with neomyci 50 mg ml nce dail for 10 days. The hairs (*H*) f intact i er hai cell are seen bov the p ll head (*P*) All uter hai cell f the two i er row (1 nd 2) ha dege erated. The remai i g cell f the third row (3) sta d at mong th collapsed ones. Phase contrast 510

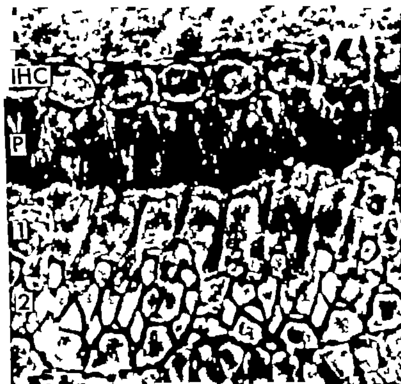


Fig. 3. G inea pig P 8 D. S rf ce preparation 2 / coils from base f cochlea 31 d intratympa ic i jection f polymyxin B 5 mg/ml. All uter hai cell ha degenerated. I the tw inner rows (1 nd 2) the coll psed cell are see distinctly but d mag t the Deiters cell has dist rted the structure f the third row Int ct inne hai cell (*IHC*) d pillar cells (*P*) Phase contrast 1300.

an idea of the pattern of the progression of the damage obtained. In this last respect a part of the material—the ears with no damage and those with complete or subtotal destruction—was of little value but in most cochleas there was a partial loss of sensory cells. In 27 cochleas a definitely localized hair cell loss was found, and on this basis these ears could be divided into three groups. In the first group (11 ears) the damage was confined to the basal (Fig 5) or basal and second turns of cochlea. In the second (5 ears) the cellular loss was limited to the apical half of the organ of Corti leaving the two lower coils intact or less damaged (Fig 6) In the third group (11 ears) the apical and basal part of the organ of Corti had suffered more cellular loss than the middle coils (Fig. 7) The pattern of degeneration thus differs from that described as a result of systemic application of ototoxic antibiotics (Kohonen, 1965)

When relating the concentrations of the applied drugs to th degree of damage it was found that the lowest concentration of neomycin, 5 mg/ml, did not cause any hi tologically discernible changes. Concentrations of 10 mg/ml and 20 mg/ml caused no damage at all in half of the ears and slight to



Fig 4 Guinea pig P 6 S. Surface preparation 2 / coil from base f cochlea Si gle intra tympanic injection of polymy in B 1 mg/ml. All sensory cell have degenerated. The general structure f the orga f Corti is still seen with collapsed i ner (*IHC*) nd outer (*OHC*) hai cells. Several ter and inner pillars (*P*) have also degenerated (between black rrows) H re the whol organ of Corti i begi ing t collapse Phase contrast 450

moderate hair cell loss in the other half Concentrations of 50 mg/ml and 100 mg/ml caused moderate to total hair cell loss in all ears. The findings were similar whether the drug had been applied by a single intratympanic injection or as "ear drops" With polymyxin B the lowest concentration of



Fig 5. G ine pig \ 16 D Singl intratympanic i jecti n of neomyci 10 mg ml The cochleogram show that hai cell loss i limited t the outer hair cell of the basal coil. Figures on left indicat dist nce from base f cochlea (1/ 1 /, 2 / 3 coil from base f cochlea) I h coil the upper row f circles represent the i ner hai cells, th lower three the outer hai cells. o——o, norm l cell •——• degenerated cells.

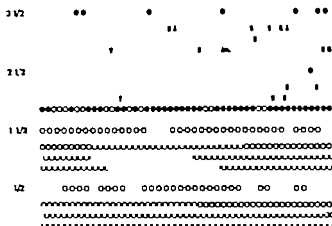


Fig. 6. Cochleogram from guinea pig N 10 D. Single intratympanic injection of neomycin 50 mg/ml. The sensory cells of the third and fourth coils of the organ of Corti have practically all degenerated, whereas the two lower coils are undamaged.

1 mg/ml appeared to be harmless, but all stronger concentrations caused definite sensory cell degeneration in each cochlea, ranging from loss of a few hair cells to complete destruction of the organ of Corti. With colimycin, the lowest concentration of 2 mg/ml left two cochleas undamaged but in six others caused slight hair cell loss. Application of solutions of 5 mg/ml and 10 mg/ml resulted in four ears in negligible damage, in eight others it caused hair cell loss ranging from slight to subtotal.

DISCUSSION

It is evident that the antibiotic solutions can reach the inner ear fluid spaces from the middle ear. This has been previously shown in animal and

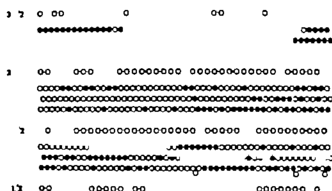


Fig. 7. Cochleogram from guinea pig P 7 D. Single intratympanic injection of polymyxin B 2 mg/ml. In the basal and apical coils almost all outer hair cells and part of the inner hair cells have degenerated, whereas the two middle coils are considerably less damaged.

clinical experiments with streptomycin (Schuknecht 1957 Spoendlin 1966) It is known that both the round window membrane and the annular ligament around the stapes footplate are easily permeable to labelled electrolyte ions and ^{131}I so it is understandable that drug solutions as well can find their way to the perilymph of the basal scala tympani and vestibuli. To reach the sensory cells of the organ of Corti they either have to penetrate Reissner's membrane into the endolymphatic space or to move directly from the perilymph of the scala tympani into the fluid space inside of the organ of Corti When speculating about the possible routes on the basis of the degeneration patterns in the present material the impression seems to be that the drugs have reached the organ of Corti via the endolymphatic space from the scala vestibuli (a) by diffusing upward along the scala vestibuli from the oval window region or (b) by travelling up the scala tympani from the round window region and round the helicotrema into the apical scala vestibuli The upward diffusion along the scala tympani would be facilitated by the upward flow of perilymph assumed to take place there (Rauch, 1966 a, b) The latter route would explain our relatively consistent finding of cochleas with the apical part of the organ of Corti far more severely damaged than the middle coils or even the middle and basal coils (Figs. 6 and 7)

The possible diffusion routes of antibiotic solutions mentioned above seem to suggest that the drugs are not able to traverse the canaliculae described by Schuknecht & Seifi (1963) to reach the interior of the organ of Corti directly from the scala tympani This would be understandable if we accepted Engström's view that the fluid space inside the organ of Corti has no channels communicating with the endo- and perilymphatic spaces (Engström 1960 Engström *et al* 1966)

The variation in the degeneration patterns as well as the varying ratio of drug dosage to degree of damage are probably explained by the admittedly inaccurate methods of application, which did not ensure equal exposure of the round and oval windows to the drugs. Neither can it be excluded that in certain cases the stapes may have been inadvertently manipulated The fact that relatively low concentrations of these antibiotics cause cochlear damage in guinea pigs does not permit direct clinical conclusions to be drawn about their use because we know that different species can vary considerably in susceptibility and must admit that the experimental conditions were not consistent with the clinical conditions. The clinical use of these drugs involves additional factors having effects which are hard to assess, e.g. infection It is possible that penetration of the drugs into the inner ear is somewhat less in infected ears (Spoendlin 1966)

We can probably accept that it is very risky to use any ototoxic antibiotics locally during ear surgery It is a more complicated question whether the use of these drugs as ear drops in chronic otitis media is associated with risk. Widespread clinical use with very few known complications suggests that the risk cannot be very large, but it is on the other hand possible that moderate hearing losses in chronically infected ears have been unduly attributed

to infection only It might be safer to consider other less toxic drugs in cases requiring prolonged local therapy for chronic otitis media

ACKNOWLEDGMENT

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ZUSAMMENFASSUNG

Experimentell erzeugte cochleare Schädigung durch intratympanale Applikati n von Neomycin, Polymyxin B und Colimycin wurde in Meerschweinchen unter sucht. Es zeigte sich, dass die erwähnten Antibiotika auch in relati niedrigen Konzentrati nen deutliche Sinnesz llschädigungen hervorrufen. Die Verä d rungen beschränkten sich meistens auf den basalen u d oder pikalen Teil der Schnecke, die Mittelwindungen des Cortischen Organs dagegen waren weniger befallen. Die möglichen Diffusionswege der Antibiotika in den Flüssigkeitsräumen des In enohres sowi das pote tial klinische Risik bei lokaler Behandlung der chronischen Otitis media werden auf Grund der Resultate diskutiert.

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X-RAY IRRADIATION OF THE INNER EAR OF THE GUINEA PIG

Early Degenerative Changes in the Cochlea

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X-ray irradiation in single doses was applied to the inner ear of guinea pigs. The organ of Corti was examined by light microscopy using the surface specimen technique as described by Engström.

1. A characteristic pattern of damage was found. The outer hair cells of the two basal coils of the cochlea were extensively degenerated, whereas the outer hair cells of the two apical coils and the inner hair cells were intact.
2. *The first sign of degeneration was coarse granulation of the nuclei.*
3. Further signs of degeneration were pyenosis of the nuclei, distortion of the normal w-pattern of the hairs and changes in the cuticular region.
4. The first sign of degeneration was found after an interval of three *hours from the exposure.*
5. Completely degenerated cells were found after an interval of six hours from the exposure
6. Degeneration was observed following X-ray doses of 4000 R and was found in an increasing scale following exposure to doses of 6000 R and 7000 R.
7. A possible genesis of the degeneration is discussed.

Damage to the sensory cells of the organ of Corti may be caused by various agents, e.g. by antibiotics (Rüedi *et al.* 1953, Hawkins, 1959, Kohonen 1965) and noise (Neubert, 1960, Engström *et al.*, 1966). In this kind of lesion the pattern of damage has proved to be characteristic. The effect of ionizing radiation on the organ of Corti has been studied earlier by histological and functional methods. Damage has been described both in the apical part (Marx, 1909) and in the basal part of the cochlea (Chilow 1927, Thielemann, 1928, Kelemen, 1963). Others report damage without giving details of its localisation (Ivanov, 1957, Berg & Lindgren, 1961). Novotny (1951) found slight impairment of the auditory function as recorded from the cochlear electrical potentials. He could not, however, demonstrate any pathological changes in the organ of Corti with histological methods. Gerstner *et al.* (1954) exposed the heads of guinea pigs to a single X-ray dose of 8000 R and examined the pinna reflex. Starting with a rise of reflex threshold 3 hours after the irradiation, the reflex was completely

abolished within 24 hours in most of the animals Histological studies were not performed. Girden & Culler (1933, 1935) however could not demonstrate any hearing impairment in dogs following cumulated doses of up to 11 000 R to the inner ear and the brain stem

The histology of the irradiated inner ears in the papers cited were performed on stained sections applying the usual light microscopy The results of these studies were controversial and motivated an investigation of the problem with a new method In the present investigation, the surface specimen technique advised by Engström (Engström *et al* 1966) was used in order to answer the following questions

1 Does irradiation of the inner ear with X rays cause any damage to the organ of Corti?
2. Do X-rays bring about any characteristic pattern of damage
3 What are the first signs of damage and how soon after irradiation are these seen?

The surface specimen technique was supplemented by examination of stained sections of the cochlea.

MATERIAL AND METHODS

Experimental animals

Twenty seven non-albino guinea pigs obtained from the same strain were used in the experiments. One of the animals died of unknown causes after the irradiation and was not included (Table 1) The histological material comprised the cochleae of the remaining 26 animals. At the start of the experiments they were approximately three months old (weight 250 g to 300 g) Only healthy animals showing a marked pinna reflex when stimulated with Bárány s noise box were used

Radiation technique and dosimetry

Technical data Siemens Stabilipan roentgen apparatus, 200 kV constant *potential, 12 mA Thoraeus filter II (0 8 mm Sn, 0.25 mm Cu 1 mm Al)* Focus—skin distance 232 mm

Table 1 *Number of animals in each group doses applied and interval between irradiation and sacrifice*

Figure in bracket represents animal dying of unknown causes.

	1000 R	2000 R	4000 R	6000 R	7000 R
1 week	1	1	2	5	7+(1)
3 hrs					3
6 hrs					4
12 hrs					3

X RAY IRRADIATION OF THE INNER EAR OF THE GUINEA PIG

Early Degenerative Changes in the Cochlea

F Ø WINTHER

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X-ray irradiation in single doses was applied to the inner ear of guinea pigs. The organ of Corti was examined by light microscopy using the surface specimen technique as described by Engström.

1 A characteristic pattern of damage was found. The outer hair cells of the two basal coils of the cochlea were extensively degenerated whereas the outer hair cells of the two apical coils and the inner hair cells were intact.

2. The first sign of degeneration was coarse granulation of the nuclei.

3. Further signs of degeneration were pycnosis of the nuclei distortion of the normal w-pattern of the hairs and changes in the cuticular region.

4 The first sign of degeneration was found after an interval of three hours from the exposure.

5. Completely degenerated cells were found after an interval of six hours from the exposure

6. Degeneration was observed following X-ray doses of 4000 R and was found in an increasing scale following exposure to doses of 6000 R and 000 R.

7 A possible genesis of the degeneration is discussed.

Damage to the sensory cells of the organ of Corti may be caused by various agents, e.g. by antibiotics (Rüedi *et al* 1953 Hawkins, 1959 Kohonen, 1965) and noise (Neubert, 1960 Engstrom *et al* 1966) In this kind of lesion the pattern of damage has proved to be characteristic. The effect of ionizing radiation on the organ of Corti has been studied earlier by histological and functional methods. Damage has been described both in the apical part (Marx, 1909) and in the basal part of the cochlea (Chilow 1927 Thielemann 1928 Kelemen 1963) Others report damage without giving details of its localisation (Ivanov 1957 Berg & Lindgren, 1961) Novotny (1951) found slight impairment of the auditory function as recorded from the cochlear electrical potentials. He could not however demonstrate any pathological changes in the organ of Corti with histological methods. Gerstner *et al* (1954) exposed the heads of guinea pigs to a single X ray dose of 8000 R and examined the pinna reflex. Starting with a rise of reflex threshold 3 hours after the irradiation, the reflex was completely

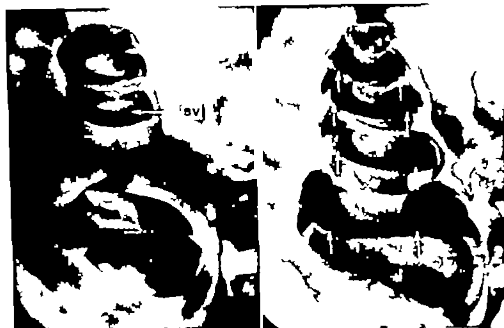


Fig 3 Left guine pig cochlea t tw stages f the preparation. ( ) The bony shell i partly removed. The stria vascularis (SV) is removed from the basal and pical coil (*b*) The bony shell nd the stria scularis removed t the tympanic side exposing the organ of Corti. Specimen for routine xaminati were taken from the rea between the arrows.

The animals were irradiated with doses of 1000 R, 2000 R 4000 R, 6000 R, and 7000 R They were sacrificed 3 hours, 6 hours, 18 hours, and 1 week after irradiation. The number of animals in each group and the doses applied are given in Table 1

Histological methods

The surface specimen technique used here is described in detail by Engström *et al* (1966)

After decapitation with heavy surgical scissors, the tympanic bullae were exposed from below by cutting away the lower jaw the remaining vertebrae and the muscles on the skull base The tympanic bullae were widely opened exposing the cochleae. The temporal bones were removed from the skull Under a binocular dissecting microscope, a hole was made in the apex of the cochlea and the round and oval windows were widely opened The fixation fluid (1.5 % veronal buffered osmium tetroxide at a temperature of approximately 4 C) was instilled through a plastic catheter (outer dia meter 0 75 mm) into the apex and allowed to flow toward the round and oval windows. To ensure rapid fixation care was taken that the procedure described above should not exceed 3 min. The temporal bones were th n placed in a small jar (2.5 ml) containing the fixative and refrigerated in



Fig. 4. Surface preparation of guinea pig cochlea 1/4 coil from the basal end. Phase contrast micrograph. *IHC*, inner hair cells. To the left the cuticular plates and to the right the hairs of the inner hair cells are in the focal plane. *P*, pillar cells; *1*, *2*, 3, first, second and third row of the outer hair cells. Cuticular plates in the focal plane. In the first and second row degenerated hair cells (*arrows*). ×1350, obj. 100, oc. 8.

darkness for 1 to 2 hours. They were then washed for 1/2 hour in physiological saline. The subsequent procedures were done under the binocular dissecting microscope. The bony capsule of the cochlea was removed (Fig. 3). The stria vascularis, the membrane of Reissner and the tectorial membrane were also removed. Samples of the organ of Corti were dissected free from the modiolus and mounted in glycerine. As a routine 1/4 to 1/3 of each of the four turns was removed, but if desired the whole organ of Corti could be mounted for microscopy. In two of the animals this was done.

"Optical sectioning" of the organ of Corti can be performed when using the phase contrast microscope and focusing the field of vision on different planes from the surface to the under side of the basilar membrane. This permits the study of different parts of the sensory cells and the supporting cells as well as the spiral vessels. Most of the animals were prepared and examined in this way.

Two of the animals were treated as described above but instead of the 1.5 % osmium-tetroxide a solution consisting of equal parts of methanol and ether was used as fixative. These specimens were then stained with Giemsas solution permitting investigation of the cell nuclei.

One animal was prepared by means of a technique described by Malllet (1963) and modified by Engstrom (Engström *et al.* 1966). The temporal

3 ½ ... lls from base of cochlea



2 ½



1 ½



½



3 ½ cells from base of cochlea



2 ½



1 ½



½



b

Fig 5 Cochleogram from guinea pig. Open circles represent cells which ... ppea ... t ... be normal. Solid black circles represent cells which are degenerated. The animal was sacrificed 1 week after irradiation. ( ) The left side, irradiated with 7000 R X rays (*b*) the right side, not irradiated.

bones were immediately after removal from the skull, placed in a petri jar containing the fixative-staining fluid consisting of three parts of 1.5% osmium-tetroxide solution and seven parts of zinc-iodide solution. The bony capsule of the cochlea was widely opened while immersed in this solution and remained immersed for 18 hours. Samples of the organ of Corti were

then prepared in the way described previously dehydrated in alcohol, cleared in xylol and mounted in canada balsam Specimens prepared in this way permit study of nerves and nerve endings as well as the sensory and supporting cells.

The cochlea of two animals were prepared for sectioning and fixed in Heidenhain Susa solution. The temporal bones were decalcified and sections of 5 μ were cut parallel to the longitudinal axis of the modiolus. Every section was mounted and stained with hematoxylin-eosin The left and the right cochlea were prepared and examined in the same way in all the animals The right cochlea served as control

Mapping the damaged sensory cells The cochleogram

The sensory cells of the cochlea form a regular geometrical pattern with one row of inner hair cells and three rows of outer hair cells (Fig 4) A schematic reproduction of this pattern is called a cochleogram (Fig 5)

Using the surface specimen technique the degenerated sensory cells can be distinguished by a characteristic appearance (Fig 4) When the sensory cell degenerates, its walls collapse inward with progressive shrinkage of the cytoplasm and simultaneous changes in the nuclear structures. At a few points the cell walls are attached to surrounding structures. The inward collapse involves the entire wall except these points, which brings about a spiderlike appearance of the degenerated cell (Engstrom *et al.*, 1966) A systematic mapping of intact and degenerated cells can be registered in th cochleogram. In this investigation a mapping was performed of approximately 40 inner hair cells and the corresponding 150 outer hair cells in each specimen.

RESULTS

Macroscopic Observations

Five of the animals which were exposed to 7000 R showed disturbance of equilibrium This will be described in a paper on the influence of X irradiation on the vestibular part of the inner ear The other animals remained in good general condition until they were sacrificed.

In all the animals a slight oedema and a slight epilation occurred in the irradiated field approximately one week after the irradiation Such changes were less pronounced in animals exposed to 1000 R and 2000 R In some cases oedema of the middle ear mucous membrane was distinct There was in no animal evidence of more pronounced ear inflammation No gross damage of the organ of Corti, the stria vascularis or the membrane f Reissner was noted during the procedure of preparation.

Microscopic Observations

Sensory cells of the right side

In the 26 cochleae prepared from the right side serving as controls, only a few scattered degenerated cells were found (Fig 5 *b*)



***Fig* 6** Surface preparation from guinea pig cochlea irradiated with 7000 R X-rays. 1 / coils from base of cochlea. Phase contrast micrograph. *IHC*, inner hair cells *1* 2, 3 first, second and third row of the outer hair cells. Focal plane at the cuticular level. Extensive degeneration of the outer hair cells. Inner hair cells apparently normal 1125, obj 100, oc. 8

Sensory cells of the left side

Observations one week after the irradiation

1000 R (1 animal) and 2000 R (1 animal) The number of degenerated cells were within the range observed in the controls.

4000 R (2 animals) In one animal an increase was found in the number of degenerated outer hair cells of the basal coil.

6000 R (5 animals) and 7000 R (7 animals) In all these animals there was extensive degeneration in the outer hair cells of the basal coil and as a rule also in the outer hair cells of the second coil (Figs 5 *a* 6 and 7) In some of the animals the degeneration comprised all the outer hair cells of the basal coil (Fig 5 *a*) In two of the animals, the whole organ of Corti was mounted for microscopy In these the degeneration proved to be continuous and not limited to the regions whence the specimens for the routine examinations were taken. The number of degenerate cells increased markedly towards the basal end of the organ of Corti In the apical direction, however the region containing the degenerate cells was sharply limited



Fig 7 Serial section (5 μ) f the I ft rgan f Corti 1 / coil from the base f the cochlea. The animal was irradiated with 7000 R X-rays nd sacrificed 1 week aft the irradiati n. Nucl i of the outer hair cells present in the first row only Nuclei f the I ner hair cells d the supporting cell present. 37L, bJ 40, oc 8.

and the outer hair cells of the upper turns did not differ from those of the controls (Fig 5 *a*) No difference in vulnerability was found between the three rows of the outer hair cells.

Only a few scattered degenerate inner hair cells were seen Even in regions where the damage to the outer hair cells was pronounced the inner hair cells appeared normal (Figs. 6 and 12 *a*)

Observations three six and eighteen hours after the irradiation

000 R (10 animals) Three hours after the irradiation, most of the nuclei of the outer hair cells of the basal coil and many of the nuclei of the outer hair cells of the second coil were coarsely granulated but had retained their shape and size (Fig 8) No changes were noted in the rest of the cell

Six hours after the irradiation, a large number of pycnotic nuclei appeared in the outer hair cells of the two basal coils (Fig 9) Also at this time many of the outer hair cell nuclei in the same region were coarsely granulated. In the cells with pycnotic nuclei, changes were also found in the hairs and in the cuticular plates. The changes ranged from a slight distortion of the normal w pattern, to complete absence of the hairs and incipient formation of a spiderlike structure in the cuticular region (Fig 10) In the same



Fig 8 Surface preparation from the basal end of guinea pig cochlea Focal plane t the level of the outer hair cell nuclei. Phase contrast micrograph. The animal wa irradiated with 7000 R X-rays nd sacrificed 3 hours fter the irradiation. *P* pillar cells; *1 2, 3* first, second nd third rows of the outer hair cells. Apparently normal nclens (*whit arrow*) nd coarsely gra ulated ncleus (*black arrow*) 1650, obj 100, oc. 10

animals an increased number of completely degenerated outer hair cells were also found. These changes increased towards the more basal parts of the organ of Corti. No changes were observed in the nerve endings (Fig. 11) Eighteen hours after the irradiation the outer hair cells of the basal coil showed severe degeneration Neither three six nor eighteen hours after irradiation was any damage to the inner hair cells seen

Supporting cells

In the areas of the organ of Corti where extensive degeneration of sensory cells was found, a breakdown of the supporting framework in the cuticular plane was also evident (Fig 12 *a*) This framework is formed by denser material on both sides of the junction of the phalanges of the cells of Deiters and the cuticular plates of the sensory cells (Iurato, 1967) The nuclei of the cells of Deiters as well as the cells of Hensen and the inner and outer pillar cells were mostly intact even in regions where an extensive degeneration of the outer hair cells were found (Fig 12 *b*)



Fig 9 Surface preparation from the basal coil of guinea pig cochlea. Phase contrast micrograph. Focal plane at the level of the outer hair cell nuclei. The animal was irradiated with 7000 R X-rays and sacrificed 6 hours after the irradiation. Pycnotic and coarsely granulated nuclei. *P* pillar cells. *1 2 3* first, second and third row of the outer hair cells. × 1350 obj 100, oc 8



a *b*

Fig 10 Surface preparation from guinea pig cochlea, lower coil from the base. Phase contrast micrograph. *1 2* first and second row of the outer hair cells. (*a*) Focal plane at the level of the cuticular plates showing incipient formation of spiderlike figure (*arrow*). (*b*) focal plane at the level of the cell showing pycnotic nucleus (*arrow*). × 1675, obj 100, oc 12.5.

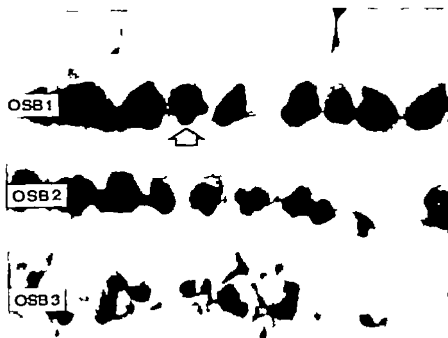


Fig 11 Surf ce preparation f guinea pig cochlea 1 / coils from the base Irradiated with 7000 R X-rays. Sacrificed 6 hours fter irradiation. Modified Maillet nerv staining. Ordinary light microscopy *OSB 1 OSB 2 OSB 3* nerv endi gs f the first, second nd third outer spiral bundles. Appare tly orm l nerv endings (*arrow*) of degenerated outer hair cell of the first row 1350 bj 100, oc 8.

DISCUSSION

Guinea pigs were chosen as experimental animals for a variety of reasons. Principally because guinea pigs have been used in much of the experimental work on the cochlea. The surgical approach to the guinea pig cochlea is simple Guinea pigs are moreover cheap and easy to handle The reason for using only coloured animals was that congenital defects in the organ of Corti may appear in albino animals (Engström, pers. comm.) The animals were approximately three months at the start of the experiments. At this age they are adults but still too young for the degenerative changes which probably occur later The hearing of the animals was tested by means of the pinna reflex. This test is far from accurate but served to exclude animals with severe hearing defects.

The 290 kV filtered X-rays ensured an almost homogenous dose distribution in the irradiated tissue with little scattered radiation. Minimal scattering of the X rays was particularly important as the distance from the midline to the inner ear is small (4 mm) The right cochlea served a control. By dosimetry the dose at th site of the right inner ear wa measured to be 4% of that at the site of the left inner ear E.g., when the left

IHC

P

OHC



b

inner ear was exposed to 7000 R, the right inner ear was simultaneously exposed to 280 R which proved to be a negligible dose in this investigation

The dosimetry was performed by means of a Baldwin Farmer Sub Standard dosimeter The reliability of the dosimeter was ensured by calibration beforehand at "Statens Institutt for Strålehygiene" in Oslo and "Statens Institut för Strålskydd" in Stockholm The ionization chamber of this instrument is a little bigger (6 mm × 20 mm) than the inner ear of a guinea pig However comparative measurements on a guinea pig phantom with this ionization chamber and with a Sieverts BG ionization chamber the size of which is about the same as the inner ear of the guinea pig showed no difference. The Sieverts BG ionization chamber is not so accurate as the ionization chamber of the Baldwin Farmers Sub Standard dosimeter and for that reason the latter was preferred. Dosimetry on a guinea pig phantom is technically difficult A phantom with dimensions corresponding to a guinea pig head was therefore constructed from hard press-wood (sp w 1 05) Measurements with the Baldwin Farmer Sub Standard dosimeter on a guinea pig phantom and on the press-wood phantom showed no difference and the final dosimetry was performed on the press-wood phantom (Fig 2)

The fixation device was constructed to prevent the animals from moving Continuous observation during irradiation showed that the device was effective Narcosis was not given because narcotics alter the radiosensitivity of tissues (Langendorff & Koch, 1954 Andrews & Brace, 1956 Alvord & Brace, 1957)

The surface specimen technique offers great advantages in an investigation like this where a survey of large areas of the organ of Corti is needed This method permits an accurate mapping of damaged or degenerated cells in relation to surrounding cells and structures. Consequently it was very useful in this study where a pattern of cell degeneration was sought for It was amazing to see how clearly structural details could be seen, a fact which made the study of early changes in the cells possible Another advantage of the method is that it is fairly little time consuming, compared with the conventional sectioning technique The specimens can be made ready for microscopic examination within three to four hours after the animal is sacrificed. It is however always of interest to have investigations performed by different methods, and in this respect conventional sectioning and staining was a valuable supplement The latter method, however is extremely time consuming and also artefacts are frequent and often difficult to distin-

Fig 12 Surface specimen from the basal coil f guinea pig cochlea. Phase contrast micrograph. The nimal was irradi ted with 7000 R X-rays and sacrificed 1 week after the irradiation. ( ) and (*b*) from the same regio of the same specimen. ( ) Focal plane t the lev l f the cuticular plates. *IHC*, inner hair cell ; *p*, pillar cells; *OHC*, region of the outer hai cells. Degeneration f all the outer hair cells with break down of the supporting framework. Inner hair cells pparently normal. (*b*) Focal plane t the lev l of the uclei of the cells f Deiters (*D*) and the outer pillar cells, (*OP*) These uclei ppear normal 1350, obj 100, oc. 8.

guish from genuine pathological alterations (Fernandez, 1958) In this investigation the surface specimen technique and the conventional sectioning technique gave the same results

Damage to the organ of Corti following irradiation with ionizing rays has been described by numerous authors (Marx, 1909 Chilow 1927 Thielemann 1928 Ivanov 1957 Berg & Lindgren, 1961 Kelemen, 1963) With the exception of Marx (1909) all the other authors call attention to hemorrhage and/or inflammatory exudate in the internal ear and suggest that the degeneration usually found is secondary to this. In some of the investigations there was also a high incidence of seropurulent exudate and hemorrhage in the middle ear (Thielemann 1928 Ivanov 1957 Berg & Lindgren, 1961 Kelemen, 1963) The bleeding tendency and the lowered resistance to infection may be caused by the effect of the whole body irradiation, affecting the reticuloendothelial system and the bone marrow This cannot be the explanation in the experiments of Ivanov (1957) and of Berg & Lindgren (1961) as the irradiation was applied to the skull and the rest of the body was properly shielded Hemorrhage in the internal and middle ear may also occur in connection with the sacrification (Engström pers. comm) No signs of bleeding or exudate in the internal or middle ear were found in our experiments. The degeneration in the organ of Corti can not be ascribed to hemorrhage or inflammation in these cases and must be due to other causes.

The lowest X ray dose causing demonstrable change in the organ of Corti was found to be approximately 4000 R. One of two animals exposed to this dose showed a slight increased number of degenerated outer hair cells. No degeneration was found in the animal exposed to 2000 R whereas a pronounced and reproducible degeneration was found in the outer hair cells of the two basal coils of the animals exposed to 6000 R A comparison of these doses with those used in the earlier investigations is difficult because no exact doses are specified (Marx, 1909 Chilow 1927 Thielemann 1928) Besides, animals other than guinea pigs were used in the experiments (Girden & Culler 1933, 1935 Berg & Lindgren, 1961 Kelemen 1963) A comparison was also difficult with earlier investigations on guinea pigs even with specified doses because the irradiation conditions differed from those in the present investigation (Novotny 1951 Gerstner *et al.*, 1954 Ivanov 1957)

Following irradiation with 7000 R, the earliest changes were found in the nuclei of the outer hair cells of the basal coil after an interval of three hours. After six hours, many of the nuclei in this area were pyknotic and after eighteen hours a great number of cells in the same region was completely degenerate This strongly supports the assumption that the nuclear changes observed after an interval of three hours from the exposure were the first sign of cell degeneration

These nuclear changes may be caused by a direct action of X rays in the cell or possibly indirectly by an altered vascular function interfering with the supply of oxygen and nutrients. A third possibility is a combination of

these factors. Devik (1955) has surveyed the theories and hypotheses explaining the possible mechanism of the local effects of radiation particularly with regard to direct and indirect effects. He also summarised earlier investigations of vascular effects induced by irradiation. He found in hairless mice that the acute epithelial skin reaction after irradiation with X rays seemed to develop independently of the state of the blood vessels, thus supporting the view of a direct action of X rays on the cells. Preliminary light microscopy of the blood vessels of the cochlea in our specimens did not reveal any major pathological changes. The functional state of the vessels however cannot be judged from histological specimens (Kreyberg, 1929). The results of a special study on the vessels of the cochlea following X-ray irradiation will be published later.

The most extensive damage was found among the outer hair cells of the basal coil of the cochlea. The damage decreased further up in the cochlea and there was usually a rather abrupt transition to normal-looking organ of Corti. While the outer hair cells were found to be extensively damaged the corresponding inner hair cells were found to be intact as studied by the present techniques. A pronounced difference in vulnerability to X ray irradiation must therefore be present between the inner and outer hair cells. There must also be a considerable difference in vulnerability between the outer hair cells of the basal and the apical coils.

Apparently for one reason or another the outer hair cells of the basal coils are in general particularly vulnerable. These cells are the first to degenerate when the cochlea is influenced by antibiotics of the streptomyces group (Hawkins & Lurie, 1953 Kohonen, 1965) and quinine (Hennebert & Fernández, 1959). Bredberg (1967) found a loss of sensory cells in the organ of Corti of elderly humans. The cell loss was particularly conspicuous in the basal coil but included both the inner and outer hair cells. After extensive venous obstruction of the labyrinth Kimura & Perlman (1956) found severe degeneration of the outer hair cells of the basal coils.

It seems reasonable to presume that the outer hair cells of the basal part of the cochlea in some way differ from the outer hair cells of the apical part. The difference may be morphological or biochemical. Bredberg (1968) found that the number of outer hair cells per square unit was higher in the basal coil than in the apical coil of the cochlea in humans. It is difficult to explain how this should be reflected in a difference in vulnerability but a parallel to this was found in the sensory epithelium of the vestibular part of the inner ear. Following exposure to X rays the degeneration was conspicuous among the sensory cells of the periphery of the maculae and cristae ampullares (Winther 1969) where Lindeman (1967) found that the cell population was denser than in the central regions. The vulnerability to streptomycin and kanamycin, however was found to be higher in the central areas than at the periphery (Lindeman, 1967).

The difference in distribution of the nerve endings perhaps also reflects a basis for the difference in vulnerability between the outer hair cells of

the basal and apical coils. The outer hair cells are supplied by two types of nerve endings, one large and richly granulated, the other small and sparsely granulated (Engström, 1958 Smith & Sjöstrand, 1961) The former type, which represents the terminations of the efferent olivo-cochlear bundle of Rasmussen (Iurato, 1962 Kimura & Wersäll 1962) are of special interest in this connection on account of their distribution In the basal coil all three rows of outer hair cells are supplied with efferent nerve endings. In the second coil the first and second row and in the two apical coils only the first row is supplied with efferent nerve endings (Engström, 1958 Smith & Sjöstrand, 1961) The pattern of degeneration found after X ray irradiation roughly corresponds with the distribution of efferent nerve endings. By the modified Maillet nerve staining technique, the nerve endings are seen as large, tuliplike clusters. Investigations performed six hours after the irradiation disclosed many completely degenerated outer hair cells in the basal coil. The clusters of nerve endings corresponding to these cells still had a normal appearance (Fig 11) Kohonen (1965) found that the size of the nerve endings of the outer hair cells decreased in radial direction from the first and innermost row towards the third and outermost row (Fig 11) Corresponding to this he found a difference in vulnerability to antibiotics of the streptomyces group in such a way that the hair cells of the first row were more vulnerable than those of the third row No such difference in vulnerability between the three rows of the outer hair cells were found in the present investigation

Biochemical differences also exist between the basal and the apical portions of the cochlea. Falbe Hansen & Thomsen (1963) found a considerable amount of a granular substance assumed to be glycogen, in the outer hair cells of the apical coils only Investigating the distribution of the oxidizing enzymes, Koide *et al* (1964) did not find any difference between the coils of the organ of Corti. In the stria vascularis and the spiral ligament however they found a higher concentration of the oxidising enzymes in the basal than in the apical coils. This points to a corresponding difference in oxygen consumption. This view was supported by the findings of Meyer zum Gottesberge *et al* (1965) They found that the oxygen consumption of the stria vascularis decreases from the basal to the apical turn The difference in metabolism of the stria vascularis may be a factor contributing to the difference in vulnerability of the outer hair cells.

The difference in vulnerability between the inner and outer hair cells is not readily explicable but both morphological and biochemical differences exist The inner hair cell have a characteristic shape with a slender upper portion a bent neck and a thicker lower portion containing the nucleus, which is bigger than the nucleus of the outer hair cell The outer hair cell is cylindrical. The inner hair cells are innervated with the great majority of the afferent neurons of the cochlea whereas only a minority are destinated for the outer hair cells (Spoendlin, 1967) The efferent nerve endings are in direct contact with the outer hair cells and are especially numerous in

the basal coils (Smith & Sjöstrand, 1961) whereas they are predominantly in synaptic contact with the afferent nerve endings or the afferent dendrites of the inner hair cells and are only occasionally found in contact with the inner hair cells (Spoendlin, 1967) Falbe-Hansen & Thomsen (1963) found the inner hair cells practically glycogen free throughout the cochlea. Koide *et al* (1964) suggested a higher content of the oxidizing enzyme succinic dehydrogenase in the outer compared with the inner hair cells. An explanation of the difference in vulnerability between the inner and the outer hair cells on the basis of these morphological and biochemical differences is not presently possible.

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ZUSAMMENFASSUNG

Die Wirkung i er Einzeldosis von Rö tgenstrahlen uf das Cortische Organ von Meerschwei chen wurde lichtmikroskopisch an Präparaten u t rsucht, die ach der Methode von Engström hergestellt waren.

1 Es konnte ein charakteristisches Degenerati nsmuster festgest llt werden. Die äusseren Haarzellen der beiden basalen Schneckenwindungen eigten ausgedehnte degenerative Veränderungen während die entsprechenden Zellen der bei den apikalen Windungen und die inneren Haarzellen normal waren.

2. Das erste Anzeichen der Degeneration war eine grobe Granulierung der Kerne.

3. Im weiteren Verlauf der Degeneration kam es zur Kernpyknose, zur Verzerrung des ch rakteristischen w Musters der Haare und Veränderungen in der Kutikularregion.

4. Di ersten Kern eränderungen wurden 3 Std. nach der Bestrahlung beobchtet.

5. Die ersten vollständig degenerierten Zellen fanden sich 6 Std. nach der Bestrahlung.

6. Di degenerativen Veränderungen wurden nach Dosen von 4000 R, 6000 R und 7000 R in st igendem Ausmass beobacht t.

7 Di Genese der beobachteten Veränderungen wurd diskutiert.

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THE INFLUENCE OF TOBACCO SMOKING NICOTINE, CO AND CO_2 ON VESTIBULAR NYSTAGMUS

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The influence of some components of the tobacco smoke on the vestibulo-ocular reflex arc was studied. In accordance with a previous report it was shown that tobacco smoking provoked an obvious increase of the vestibular nystagmus frequency while the speed of the slow components remained unchanged. The injection of nicotine intravenously gave a similar change in the nystagmus pattern as was obtained after smoking An increase in the carboxy hemoglobin content by smoking a nicotine-free cigarette did not change the vestibular nystagmus pattern An elevation of the arterial carbon dioxide tension affected the vestibular nystagmus only slightly and in an opposite way compared to smoking The results indicate that nicotine is the main component in the tobacco smoke responsible for the changes in the nystagmus pattern observed after smoking. There is evidence that the effects observed are due to changes in neuronal transmission of the vestibulo-ocular reflex arc.

The gas phase of tobacco smoke contains several substances (Osborne *et al* 1956) of which nicotine carbon dioxide (CO_2) and carbon monoxide (CO) are known to influence cerebral function Each of these agents has been claimed to have effects on the vestibulo-ocular reflex arc (Klein & Verstegh, 1922 Noveling & Kruse, 1961 Gellhorn & Spiesman, 1935 Floberg, 1953) on the cerebral blood flow (Kuhn, 1967 Schmidt, 1950 Lassen, 1958) and on different central nervous processes (Longo *et al.*, 1954 Metz, 1960 Lilienthal 1950)

In a previous report, it was shown that the vestibular nystagmus pattern changed immediately following cigarette smoking (Tibbling & Henriksson, 1968) The fast phase of the nystagmus beat was selectively influenced. As suggested by McCabe (1965) the reticular formation is involved in the production of the fast components. It was thus concluded that the changes observed after smoking were centrally evoked

The aim of the present investigation was to study whether the nicotine CO_2, or CO content of the tobacco smoke is responsible for the changes in vestibular nystagmus observed after smoking The per rotatory nystagmus pattern was therefore studied in subjects exposed to a vestibular test after (1) smoking a conventional cigarette (2) after administration of CO by

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smoking a nicotine-free cigarette (3) after intravenous injection of nicotine and (4) after inhalation of CO_2. The nystagmus pattern from repeated vestibular tests was used as a control

MATERIAL AND METHODS

The study included 25 healthy subjects (15 males, 10 females) aged 15–28 years. The subjects in *group A* ($n = 10$) participated in the nicotine-containing (A_1) and nicotine-free (A_2) *smoking tests*, and in the CO_2-inhalation test (A_3) *group B* ($n = 5$) in the nicotinecontaining smoking test (B_1) and the nicotine-injection test (B_2) and *group C* ($n = 10$) the control subjects, in repeated vestibular tests (Fig 1) Everyone who participated in a smoking test was a habitual smoker with a consumption of at least 10 cigarettes per day Before each experiment, which was performed in the morning, the subject had a twelve-hour discontinuance of smoking No drugs (sedatives) and no alcohol were allowed for 24 hours prior to the tests

The vestibular test

The subjects were sitting in a rotation chair in total darkness and were ordered to keep their eyes open, and to report about perception of rotation and non rotation. They were subjected on an angular rotation with the horizontal canals of the labyrinths placed at right angles to the vertical axis of rotation and with constant velocity after an acceleration of 120 /sec in 1.8 seconds. The rotation lasted for 1 minute The vestibular test was performed before, immediately after and 15 minutes after the administration of the different agents to be tested (Fig 1) In the control group the vestibular test was repeated twice with intervals of 5 minutes.

The smoking tests

The smoking tests were carried out with either a conventional cigarette without filter (John Silver® Swedish Tobacco Company Sweden) giving a mean content in the smoke of 1 7 mg nicotine per cigarette (Kungl. Medicinalstyrelsen, 1965) or with a nicotine-free cigarette made from the leaves of lettuce (Bravo® Arten Lactura Sativa, Texas, USA) in order to obtain an elevation of the COHb-level During the 3-minute smoking period the subjects were ordered to take a deep inhalation of smoke every 15 seconds. About three quarters of the cigarette were consumed. The subjects were not aware of what sort of cigarette he was smoking until the study was finished The vestibular test started within 10 seconds after discontinuance of smoking.

The carboxyhemoglobin (COHb) level in venous blood before and after the smoking tests (Fig 1) was determined by a gas chromatographic method (Ehrner-Samuel & Overum, 1969) The precision of the method with blood samples containing 1–2 g of COHb per 100 ml was ± 0.02 (± 1 S.D) Th content of COHb averaged 1.6 g per 100 ml after 12 hours discontinuance of

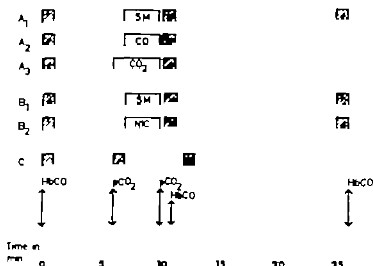


Fig 1 Schemati diagram f the design f the study *A B* nd *C* refer t different groups f test subjects. A_1, A_2 nd A_3 well s B_1 nd B_2 were carried out different d ys, whil *C* wa mad in session. *SM* represents smoki g test (icotine-co taini g cig rett) *CO* represents admi istrati n f CO by smoki g a nicoti e-free cigarette, *CO* the inhalati n f CO_2, and *NIC* the injection f nicotine Arrows represe t blood samples taken n subjects i groups A nd B.

smoking, i e it was elevated in comparison to the normal range of 0 1–0.5 g per 100 ml in non smokers (Henry 1964) After smoking the nicotine containing cigarette, the COHb level increased to 2 4 g per 100 ml This mean increase of 0 8 g per 100 ml was of the same magnitude as that after smoking the nicotine-free cigarette (Table 1) Thirty minutes later the COHb con centration had not returned to the level before smoking The increase of the COHb-level was used as a criterion of the ability of the subject to inhale the smoke. In one subject no change in the nystagmus pattern and no increase in the heart rate were obtained after smoking This subject was the only one having a normal basal level of COHb There was no increase in COHb-concentration after smoking a nicotine-containing nor after a nicotine-free cigarette For that reason this subject was excluded from the results and replaced by another

The nicotine injection test

A nicotine tartrate solution of 1 mg/ml was used An initial test dose of 1–2 mg was given intravenously during three minutes. This dose was too low to get significant change of the nystagmus pattern The test subjects were then re-examined on another occasion with a dose of nicotine varying from 3 to 6 mg

CO_2 inhalation test

Elevation of the arterial CO_2 tension was brought about by the inhalation of a CO_2-containing gas mixture through a semiopen non rebeathing valve

Table 1 *Carboxyhemoglobin in gram per 100 ml before and after smoking*

Mean values are given with standard erro f the mean and ranges.
p-values refer t comparison between al es before and after smoking.

Sort f cigarett	Before	T m fer smoking		
		1 m n. fter	15 min. after	30 min. after
J hn Silver® $n=8$	$1\,73 \pm 0.22$ (0.7 2.5)	2.49 ± 0.21 (1.5–3.4) $p < 0.001$	—	2.03 ± 0.20 (1 1 2.6)
Bravo® $n=8$	$1\,88 \pm 0.37$ (0.8–4 1)	2.60 ± 0.29 (1 6–4.2) $p < 0.001$	2.22 ± 0.36 (0 7–4.1) $p < 0.05$	

system during 4 minutes. The gas mixture contained 7% CO, 30% O_2 and 63% air The inhaled CO_2 content which was checked with conventional Scholander analysis was about ten times the content of CO in the air cal culated to reach the alveoli by smoking a cigarette (Philips, 1956) Within 5 seconds after the CO_2-inhalation was stopped, the vestibular test was started

The effects of CO_2-breathing on the arterial CO tension was controlled in a separate study in order not to infere with the test situation. The pCO_2, pH, standard bicarbonate, and base excess were determined on capillary blood samples by conventional "micro-Astrup" analysis (Radiometer Copenha gen) The mean rise in pCO_2 during CO_2-breathing of the type used in group A was 14 mm Hg (from $53 \pm 1\,0$ to 50 ± 2.5) Simultaneously the pH decreased. The standard bicarbonate and the base excess values were un changed.

Recordings

The horizontal eye movements of the subjects were reco ded by means of a DC-technique previously described (Lundgren *et al* 1969) and the horizontal head movements by means of a potentiometer arrangement on the head (Henriksson *et al* 1967) Before starting the rotation a calibration of the horizontal eye and head movements for twenty five degrees from the mid-point was made During the whole smoking procedure half a minute before rotation, and during the whole rotation test, the pulse was registrated from a finger by means of a photo cell technique (Elema Schönander Sweden) The angular velocity of the rotating chair was recorded by means of an optical device All recordings were fed to a 4-channel ink writer (Mingograf 81 Elema Schönander Sweden) which was used with a paper speed of 2.5 cm/sec

Analyses of the tracings

The per-rotatory tracings at every vestibular test were analysed for the f llowing qualities frequency of nystagmus beats per first 10 seconds

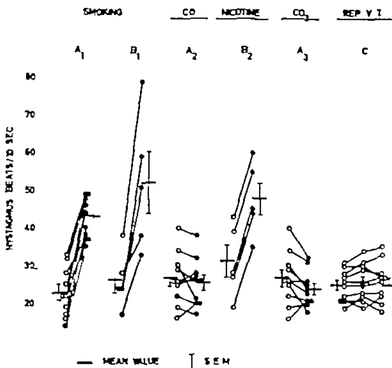


Fig Mean nystagm frequency during the first ten seconds f rotatio in different test 1 given with standard erro f the mean. Individual nystagmus frequency valu bef re (O) nd after dministration of an gent (●) *Rep. V.T* = repeated vestibular tests. *A B*, nd *C* refer to differe t group f test subjects.

(Tibbling & Henriksson, 1968) of rotation, speed of the slow components 2 4 10 18 and 30 seconds after start of rotation (each value represents the mean value of three consecutive nystagmus beats) duration of nystagmus and duration of sensation of rotation. The heart rate per minute was deter mined from the pulse curves. The differences in the nystagmus patterns *between the tests were statistically evaluated by means of Wilcoxon s rank* sum test (Documenta, Geigy 1962)

RESULTS

1 After smoking a conventional cigarette the nystagmus frequency in group A changed from a mean of 24 to 44 beats/10 sec ($p < 0.001$) and in group B from 27 to 53 beats/10 sec. This corresponded to a frequency in crease of 90% and 95% respectively Fifteen minutes later it was reduced *to 40% and 12% respectively*

2. No change in the nystagmus pattern was obtained after administration of CO by smoking the nicotine free cigarette (28 beats/10 sec before and 27 beats/10 sec after) (Fig 2)

3 Following the injection of nicotine the mean nystagmus frequency increased from 32 beats 10 sec to 49 beats/10 sec ($p < 0.02$) (Fig 2) This

Table 2 *Cardiac rate per minute following tobacco smoking injection of nicotine inhalation of CO_2 and administration of CO by smoking a nicotine-free cigarette*

Mean values are given with standard error of the mean. *p*-values refer t comparison between values obtained before and after administration f the gent t be tested.

	Before	Immediat ly following	15 min. after
Smoking			
Group A_1	80.9 ± 5.2	109.3 ± 4.9 $p < 0.001$	87.7 ± 5.8 $p < 0.05$
Group B_1	77.8 ± 3.5	98.4 ± 7.4	83.0 ± 6.0
Nicotine injection			
Group B_2	74.8 ± 2.3	83.2 ± 2.7	77.2 ± 3.2
CO_2-inhalation			
Group A_2	75.6 ± 4.0	85.8 ± 4.7 $p < 0.01$	81.4 ± 4
CO			
Group A_4	81.4 ± 4.7	82.6 ± 4.1	73.3 ± 4.0

corresponded to a frequency increase of 56% which fell to 10% 15 minutes later

4 Inhalation of CO_2 caused a slight decrease of the nystagmus frequency from a mean of 28 beats/10 sec before inhalation to 25 after This decrease of the nystagmus frequency was significantly different ($p < 0.02$) from the small changes obtained in repeated vestibular tests without smoking (Fig 2)

During the first 10 seconds of the vestibular test in control group C the nystagmus frequency averaged 26 beats. When the vestibular test was repeated twice with 5-minute intervals, no significant changes were obtained between the three mean values (Fig 2)

Other variables in the nystagmus curves, such as the speed of the slow components, the duration of the nystagmus after start of the rotation, and the duration of the per rotatory sensation were also measured but did not vary significantly between the tests.

The recorded control of the head movements showed that no changes of the head position took place during the vestibular tests.

Heart rate

The influence of smoking on the heart rate varied in proportion to the influence on the nystagmus pattern. After smoking a nicotine-containing cigarette (1) the pulse increased from a mean of 81 beats/min to 109 (+35%) in group A and from 78 to 98 (+26%) in group B (Table 2) The time for normalization of the cardiac rate was 20–30 minutes The admini

stration of nicotine intravenously (2) caused a mean increase from 75 to 83 beats/min (+11%) Smoking a nicotine-free cigarette (3) caused an elevation in the COHb concentration. This increase was not followed by any change in the heart rate, which was in accordance with the unchanged nystagmus pattern. The CO_2-inhalation (4) caused a change of the heart rate from 76 to 86 beats/min (+13%) It was not fully normalized 15 minutes later

DISCUSSION

In a previous report it was shown that tobacco smoking, immediately before a standardized vestibular stimulus, causes a change in the nystagmus pattern (Tibbling & Henriksson, 1968) The fast phases, generally believed to be determined by central nervous mechanisms, were selectively influenced. Estimation of the nystagmus frequency per 10 second was found to be the best parameter for evaluating the degree of change In the present report the influence of cigarette smoking on the vestibular nystagmus pattern was confirmed

After smoking a nicotine-free cigarette the change in the nystagmus frequency failed to appear Since the rise in COHb content was the same after smoking a conventional cigarette as after a nicotine-free one, it must be concluded that the increase in COHb is not responsible for the change in the vestibular nystagmus pattern induced by tobacco smoking

After administration of nicotine intravenously a similar change in the nystagmus pattern was achieved as after inhalation of the smoke from a nicotine-containing cigarette The influence on the nystagmus pattern was, however less than after smoking the cigarette, although the injected quantity of nicotine was about five times greater than the quantity supposed to be resorbed by smoking This discrepancy in dose-response, also demonstrable in the cardiac rate may be explained by the difference in administration of the nicotine It is not however excluded that some other components beside nicotine may be included in the effects of tobacco smoke or that such component, or components, may potentiate the effect of nicotine on the vestibular nystagmus pattern

After an elevation of the arterial pCO_2, a slight decrease in the nystagmus frequency was seen This decrease, however would counteract the increase observed after smoking After the termination of the CO_2-inhalation the pCO_2 drops very quickly and returns to normal values within about one minute The normalization of the cerebral blood flow is however delayed for about half a minute (Shapiro *et al* 1965) In the present investigation the nystagmus pattern was found to be uninfluenced even before the pCO_2 had returned to normal level It may therefore be concluded that neither the CO_2 content of the tobacco smoke nor a supposed increased cerebral blood flow could have been responsible for the influence on the nystagmus pattern after smoking

The present results show that nicotine appears to be the main agent in the tobacco smoke responsible for the influence on the vestibular nystagmus pattern The effect of nicotine on central nervous structures of different animals has been experimentally well established (Armitage *et al* 1967 Bradley & Wolstencroft, 1967) Following the demonstration of Longo *et al* (1954) that nicotine induces desynchronization followed by seizure activity in the electrocorticograms in rabbits, nicotine has been widely used as a pharmacological tool in the research on central nervous processes, mainly in animals. Yamamoto & Domino (1965) showed in cats that nicotine gives an arousal response of the EEG. In man nicotine exerts peripheral effects on autonomic ganglia and neuro-effector junctions, and on the cardio-vascular system, probably by releasing catecholamines (Volle & Koelle, 1967) It appears possible that nicotine exerts similar effects on the nervous transmission in the CNS and, hence has an influence on central parts of the vestibulo-ocular reflex arc.

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ZUSAMMENFASSUNG

Der Einfluss ei iger Inhaltsstoff des Tabakrauches auf den vestibulo-okularen Reflexbogen war Gegenstand der Untersuchung. In Übereinstimmung mit einer orangegangenen Arbeit kann gezeigt werden, dass Tabakrauch eine offensicht lich Steigerung der Nystagmusfrequenz hervorruft, während die Geschwindigkeit der langsamen Phase unverändert bleibt. Ähnliche Veränderungen des Nystagmusmusters können auch durch di intravenöse Verabfolgung v n Nikotin usgelöst werden. Die Vermehrung des Blut-Carbo y Hämoglobin-Gehaltes durch Rauchen nikoti freier Zigaretten verändert das vestibuläre Nystagmusmuster nicht. Eine Er höhung des arteri llen Blut Kohlendioxyd-Gehaltes führt nur zu einer geringfügi gen Änderung des vestibulären Nystagmus, und zwar in entgegengesetzter Richtung zu der durch Tabakrauch ausgelösten. Diese Ergebnisse deuten daraufhin, dass Nikotin im T bakrauch hauptsächlich für die beim Rauchen beobachteten Ver ä derungen im Nystagmusmuster verantwortlich ist. Es ist anzunehmen, dass diese Beoba htungen auf Veränderungen im Bereich der neuronalen Übertragung im vestibulo-ok laren Reflexbogen zurückzuführen sind.

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RELATION BETWEEN STRENGTH OF ACCELERATION AND DURATION OF POSTACCELERATORY NYSTAGMUS

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Altogether 22 clinically healthy subjects were rotated at acceleration strengths of 1 2, 4 and 8 /sec² After each acceleration the duration was measured of postacceleratory nystagmus, denoted as the *after-discharge* The after-discharge showed a distinct tendency to be longer with increasing strength of stimulus, although a relatively small increase was noted in the acceleration range 4–8°/sec² On the average, the after-discharge was approximately the same in clockwise and in counterclockwise acceleration. When a difference was present, it was generally greater after weaker stimuli, but large i dividual variations exist in normal subjects, nd their borderlines were therefore calculated. The investigation also disclosed that the results are not in agreement with those found in move ment of a damped pendulum. After stronger stimuli, a shorter after discharge was recorded than that one would have expected had this applied.

In the early 1900s, the following test method was used for evaluating the vestibular reactions elicited by rotation. The patient was first accelerated strongly to a certain speed, this acceleration being ended by abrupt braking to a standstill after which the nystagmus was studied. This is denoted as Bárány s test.

When electrically driven rotation chairs had been constructed, the patient could be slowly accelerated to different constant speeds, followed by abrupt braking, i.e., cupulometry (van Egmond *et al* 1948)

In both these test methods, a deviation of the cupula occurs during brak ing, followed immediately by its return to a neutral position. When the cupula deviates, a rapid change is brought about in the flow of impulses in the ampullar nerve A return to spontaneous activity then follows in association with the cupula s return to the resting position. These variations in the impulses in both labyrinths are transmitted to the nuclei of the eye muscles, as well as to synapses in the reticular formation, and nystagmus results (Schmaltz, 1925 van Eyck, 1955 Trincker 1962)

A simpler course is produced from the neurophysiological point of view if one lets a constant rotatory acceleration be ended by a transition to a constant speed. During the constant acceleration, the cupula has assumed a certain position. After the end of acceleration, it returns to the neutral posi

tion. The greater is the deviation in the initial position of the cupula, the longer is its time of return This implies that strong acceleration should elicit nystagmus of longer duration than that after weak acceleration. The possibility thus exists of studying how the duration of nystagmus is changed when the time of return of the cupula is varied by using different strengths of stimulus. We have denoted the time from the cessation of acceleration to the last beat of the nystagmus reaction as the *after-discharge* A study of the duration of the after-discharge following 4 $/sec^2$ acceleration has recently been published (Collins & Guedry 1967) A mean value of about 30 sec was noted. Since the after-discharge at different strengths of stimulus was not the direct object of the investigation, no data are given on this matter

As we were unable to find any information about the after-discharge at different strengths of stimulus, the study reported in the following was undertaken.

CASE MATERIAL

Altogether 44 subjects of both sexes were studied. Their age ranged from 20–45 years. About one-third were medical students and nurses, and the remainder consisted of patients from the Ear Nose and Throat Clinic. All were in good physical and mental condition, and none had any disease that could be envisaged to influence the results. All had normal hearing, and none had taken any medicine of any kind on the days preceding the examination.

APPARATUS FOR STIMULATION

For the investigation, we used a rotation chair (Stille Werner modification Fluur) in which the subject sat with his head secured in a holder and inclined 30 forward It was checked that the chair s axis of rotation was directed on a point halfway between the subject s ears. A special stand fixed to the ceiling was used for this purpose

The chair could be accelerated and decelerated with known stimuli, adjustable on a dial, in $/sec^2$ from 0 1 to 10 0 $/sec^2$ and the speed kept constant for a given time. The speed of the chair could be read off constantly on a galvanometer

METHOD OF EXAMINATION

The experiments were divided into two groups, comprising the following strengths and durations of stimulation

Group 1	1 $/sec^2$ for 40 sec	*Group 2*	4 $/sec^2$ for 30 sec
	2 $/sec^2$ for 40 sec		8 $/sec^2$ for 20 sec
	4 $/sec^2$ for 30 sec		

The examination was carried out as follows. The subject sat in the rotation chair and the electrodes and lightproof diving mask were put on The

procedure was explained to him, and a test acceleration was made at about 2 /sec^2 to check that everything was functioning The subject was also in formed about the nature of the experiment

One experiment in group 1 started from a constant speed of about 5 /sec^2 after which we accelerated clockwise at 1 /sec^2 for 40 sec, followed by a constant speed for a few minutes. The chair was then decelerated at 1 /sec^2 for 40 sec, again succeeded by a constant speed for a few minutes. This acceleration–deceleration test was repeated twice more to permit a study of the reproducibility of the measurements in a later paper This was followed by stimulation at 2 /sec^2 for 40 sec and at 4 /sec^2 for 30 sec, in exactly the same way as at 1 /sec^2

The experiments were made in a similar way in group 2, at 4 /sec^2 for 30 sec, and 8 /sec^2 for 20 sec.

RESULTS

A good idea of the results is given by Figs 1 and 2, which comprise a graphic representation of the results tabulated in Table 1 The digits denote the duration of nystagmus after the end of acceleration (Fig. 1) and dece leration (Fig 2)

Fig 1 shows the results of measurements in clockwise acceleration It is seen that at 1 /sec^2 the after-discharges ranged from 7 to 75 sec (mean 23.5 sec) At 2 /sec^2 the values are in the time range 6–54 sec (mean 27.3 sec) The increase from 1 to 2 /sec^2 produced a prolongation of the after discharge in 14 subjects, whereas in the others it was unchanged or shorter Acceleration at 4 /sec^2 resulted in a mean after-discharge of 29 sec (range 17–53 sec) The increase from 2 /sec^2 to 4 /sec^2 produced a prolongation of the after-discharge in 13 subjects. The accumulation of dots for 8 /sec^2 lay in the range 21–41 sec (mean 31 sec) A prolongation of the after-dis-

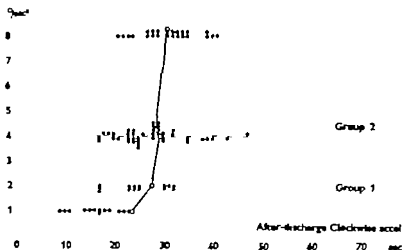


Fig 1 Relation between strength f stimulus nd d ration f fter-discharge clockwise ccel ration.

Table 1

Acc.	Case 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Group 1. *Clockwise acceleration* (1 /sec² 2°/sec² and 4 /sec²)														
1	21	7	9	75	35	12	11	27	15	17	30	23	22	19
2°	25	21	6	41	38	23	17	54	31	24	30	17	17	32
4	30	17	21	30	25	23	23	53	35	28	25	25	24	40
Counterclockwise acceleration														
1	9	23	8	18	26	29	19	31	6	17	24	38	10	24
2	23	30	16	23	23	34	26	47	27	18	27	27	28	28
4	28	24	32	28	25	32	26	57	42	22	24	30	29	31
Group 2. *Clockwise acceleration* (4 /sec² and 8°/sec²)														
4	29	38	28	29	30	32	28	24	29	29	18	32	23	28
8°	28	35	27	22	24	35	29	23	31	32	31	21	27	40
Counterclockwise acceleration														
4	29	32	24	17	33	33	30	33	41	31	30	35	21	24
8°	19	34	26	20	34	28	27	34	29	41	31	27	33	29
Group 1. *Difference between after-discharge in clockwise and counterclockwise acceleration*														
1	+12	−16	+ 1	+57	+ 9	−17	−8	− 4	+ 9	±0	+ 6	−13	+12	− 5
2°	+ 2	− 9	−10	+18	+15	−11	−9	+ 7	+ 4	+6	+ 3	−10	−11	+ 4
4	+ 2	− 7	−11	+ 2	± 0	− 9	−3	− 4	− 7	+6	+ 1	− 5	− 5	+ 9
Group 2.														
4	± 0	+ 6	+ 4	+12	− 3	− 1	−2	− 9	−12	−2	−12	− 3	+ 2	+ 4
8	+ 9	+ 1	+ 1	+ 2	−10	+ 7	+2	−11	+ 2	−9	± 0	− 6	− 6	+11

charge occurred in 12 subjects when the strength of acceleration was increased from 4 to 8 /sec²

In counterclockwise acceleration, the results were as follows (Fig 2) At 1 /sec² the after-discharge had a range of duration of 5–36 sec (mean 21.2 sec) An increase to 2 /sec² gave values from 12–47 sec (mean 25.8 sec) At this strength of stimulus, a prolongation was noted in 16 subjects. When the strength was increased to 4 /sec² the duration ranged from 11–57 sec (mean 29.2 sec) and a prolongation of the after-discharge was recorded in 14 subjects At 8 /sec² the mean value was 30.2 sec (range 19–43 sec) The increase from 4 to 8 /sec² produced an increase in the after-discharge in 15 subjects.

It is seen that at 1 /sec² the accumulations of dots representing clockwise and counterclockwise acceleration are spread over a fairly large time range This applies particularly at 1 /sec² clockwise acceleration, where the value of 75 sec deviates greatly from the others. At the higher strengths of stimulus, the accumulations of dots lie closer together This tendency is especially marked at 4 and 8 /sec² and is about the same at both these

15	16	17	18	19	20	21	22	$\bar{X}$	S	C	$K \cdot S$	$\bar{X} \pm K \cdot S$	
14	10	41	32	16	17	18	47	23.5	15.56	0.66	41 97	−18 43	+65.51
30	16	24	23	35	40	26	32	27.3	10.56	0.30	28.48	− 1.21	+55.75
39	25	20	42	17	43	24	38	29 4	9 81	0.32	25.63	+ 3.76	+53.06
6	29	26	32	21	18	27	28	21.2	8.95	0.42	21 14	− 2.96	+45.32
12	17	38	13	21	29	35	25	25.8	8.21	0.32	22.22	3.55	+47.99
22	23	32	11	18	36	22	36	28.6	9.22	0.32	24.87	+ 3.77	+53.51
19	23	47	21	20	26	45	28	28.6	7.28	0.25	19.63	+ 8.96	+48.22
33	34	39	31	33	41	30	28	31.0	5.80	0 19	15.61	15.40	+46.68
40	25	41	26	21	25	34	24	29.8	6.45	0.22	17 40	+12.42	+47.22
43	27	33	28	36	32	41	22	30 2	6.31	0.21	17.02	+13.16	+47.20
9	−19	+16	± 0	− 6	− 1	− 9	+19	2.36	16 29		43.91	−41.55	+46.27
18	− 2	−14	+10	+14	+11	−10	+ 7	+1.50	10.52		28.37	−26.87	29.87
17	+ 2	−12	31	− 1	+ 7	+ 2	2	0 77	9.62		25.95	−25.18	26.72
−21	− 2	6	− 2	− 4	+ 1	9	2	1.23	7 45		20.00	21.32	18.86
10	+ 7	+ 6	+ 3	− 7	+ 9	− 2	+ 6	0.86	6.85		18.47	17 61	+19.33

strengths The results after stimulation at 4 /sec² are fairly similar in the two groups.

Thus, the values were less scattered with a longer duration of the after discharges, which was associated with stronger stimulation. In Table 1 this is illustrated by the C values, which decrease from 0 66 and 0 42 at 1 /sec² to 0 19 and 0.21 at 8 /sec² $C = S/X$ (coefficient of variation = standard deviati n/arithmetic mean)

To illustrate further the scattering of the results, we also calculated the borderline values in such a way that 95% of normal subjects would be in cluded with 95% certainty These values were calculated according to a formula in which bo derline values are denoted by $\bar{X} \pm$ constant $K \cdot S$ These ha e been inserted on the right in Table 1 At 1 /sec² it is seen e.q that clockwise acceleration can produce after-discharges which are regarded as normal within the time range 0–65.51 sec The corresponding time range for 8 /sec² clockwise acceleration is 15 40–46 68 and thus comprises a time range of 31.28 sec.

It is also evident from Tabl 1 that the upper borderline values were not

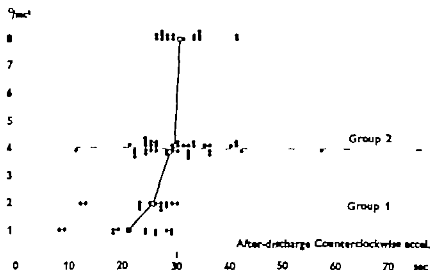
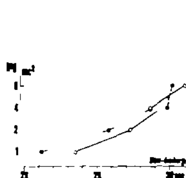


Fig 2 Rel ti n between strength f stimulus nd duration of fter-discharge counter lockwise acceleration.

definitely displaced in either direction when the strength of stimulus was increased The lower borderline values, on the contrary increased distinctly when this was done.

We also studied the way in which the after-discharges in the individual subjects were related to clockwise and counterclockwise acceleration, respectively In several subjects, the duration was approximately the same after stimulation in both directions, whereas large differences were noted in others At 1 /sec² the differences ranged from +57 to −19 sec. This implies that in the former case, the after-discharge was 57 sec longer after clock wise acceleration than after counterclockwise, whereas in the latter it was 19 sec longer after counterclockwise acceleration than after clockwise The


F g 3.

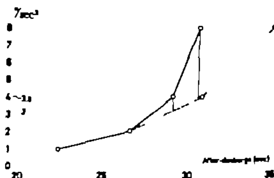


Fig 4.

Fig 3 R l tion between l g strength f stim l s nd duration f fter-discharge ——— Clockwise ccelerati – – – co nterclockwise ccelerati n.

Fig 4 R l tio between strength of stim l nd rithmetic mea f d ration f fter discharg in both clockwise nd counter-clockwise cceleration. F expl nation f broken line, see Discussion, p 133

mean difference is +2 4 sec. Stimulation at 2 /sec² resulted in a clockwise–counterclockwise difference ranging from +18 to −11 sec (mean +1.5 sec) After stimulation at 4 /sec² differences ranging from +31 to −11 sec were recorded in group 1 and from +12 to −21 sec in group 2. The mean difference is +0 77 and −1.23 sec, respectively At 8 /sec² stimulation, the range of differences was from +11 to −11 sec (mean +0.86 sec)

On the basis of these results, we also calculated the borderline values applying for 95% of normal subjects to be included with 95% certainty These borderline values thus apply for the difference between the after discharge in clockwise and counterclockwise acceleration, respectively They are apparent from the following tabulation

1 /sec²	46.3 to −41 6 sec
2 /sec²	29 9 to −26.9 sec
4 /sec²	26 7 to −25.2 sec
8 /sec²	19.3 to −17 6 sec

We wished to ascertain whether the successive prolongation of the after discharge on strong stimulation observed in Figs. 1 and 2 did, in fact, follow the exponential course typical of sense organs. Consequently the mean values of the after-discharges were plotted in Fig 3, together with the strength of stimulus on a logarithmic scale The curve for counterclockwise stimulation is seen to be almost linear from the 1–4 /sec² level whereas it subsequently has a much steeper course The curve for clockwise stimulation does not have a completely linear course but a successive increase in the slope takes place within the whole acceleration range Thus, the after-discharge does not seem to be in linear correlation to the logarithm of stimulus.

Finally Fig 4 illustrates the average values of the after-discharge in both clockwise and counterclockwise acceleration (unbroken line) It is seen that the duration of the after-discharge increases by 4.2 sec when the strength of stimulus is raised from 1 to 2 /sec² The next change—i.e., to 4 /sec²—produces only a 2.5 sec prolongation of the after-discharge When the stimulus is increased to 4–8 /sec² the prolongation amounts to 1.5 sec

DISCUSSION

It is seen in Figs. 1 and 2 that, when the strength of acceleration is increased, the accumulation of dots is displaced to the right, but that this displacement is far less on an increase in the 4–8 /sec² range in counter clockwise acceleration, as well as at 2–8 /sec² in clockwise acceleration. It thus appears as if use of stimuli above 2–4 /sec² does not result in any markedly longer after-discharges than does stimulation at 2–4 /sec

Fig. 1 discloses that the scattering of the accumulation of dots is fairly large at 1 and 2 /sec² but that thereafter they lie increa ingly closer together This occurs concurrently with an increase in duration of the after discharges. This implies that weak acceleration elicits nystagmus which

seems to produce greater variations in the duration than does the nystagmus elicited by strong stimuli.

If the mean values in the groups thus show an increase in duration of the after-discharge with rising strength of stimulus, the question arises whether each individual consistently follows the same course. It can be inferred from the individual results listed in Table 1 that this applies to only a limited number of cases. In group 1 such a successive increase can be noted in 7 subjects in clockwise acceleration and in 10 in counterclockwise. It therefore appears as if the tendency to a longer after-discharge is distinct on increasing strength of stimulus, but that the individual measurements vary in such a way that displacements to both a longer and a shorter duration occur An important observation is that, in just over half of the 22 subjects, an increase took place in the duration of the after-discharge when the strength of stimulus was doubled This even applies to group 2, in which the numerical values suggested an inappreciable increase in duration. This strengthens the observation that a prolonged after-discharge did actually occur in the 4–8 /sec² range as well, despite the displacement of the accumulations of dots in Figs. 1 and 2 being apparently small.

The calculated borderline values given on the right in Table 1 show that a long after-discharge of more than 46–55 sec can normally occur in the whole acceleration range of 1–8 /sec² On the other hand acceleration at 4 /sec² and particularly at 8 /sec² should normally produce after-discharges of 3 7 sec and more.

The differences between the after-discharge in clockwise and counter clockwise acceleration demonstrate that large individual variations exist The mean differences are numerically largest at 1 and 2 /sec² At these strengths of stimulus, the scattering of the accumulations of dots in Figs. 1 and 2 was, in fact, greatest. In both cases, the probable explanation is that acceleration at 1 and 2 /sec² elicits such weak nystagmus that it is easily influenced by the central nervous system. The stronger stimuli (4 and 8 /sec²) showed smaller differences between clockwise and counterclock wise acceleration. As far as the size relation clockwise-counterclockwise is concerned, the values of the mean differences are so small as to permit the statement that the after-discharges are about the same in both directions. Here the constant variations in the excitability of the reflex arc play a role, and are responsible for occasional values of such an order of magnitude that a pronounced difference between the directions seems to exist.

Our borderline values for both the after-discharges and the differences between those after clockwise and counterclockwise stimulation are in fact purely mathematical ones. They should not be applied too strictly to a biological process, but should rather be regarded as directives for evaluating the reactions in the individual case

If the individual results are studied one can actually find a few subjects with a consistently strong decrease in the after-discharge in one direction, despite an increased strength of stimulus. In these cases, this tendency was

not present with respect to the after-discharges in the opposite direction It cannot be stated whether these observations are to be interpreted as a sign of fatigue or of habituation, or whether they are merely an expression of changes in the vestibulo-ocular reflex are dependent on the central nervous system.

Since this investigation concerned a reflex arc elicited through deviation of the cupula, the question arose whether the special properties of the cupula are reflected in the whole mode of reaction of the nystagmus reflex. It is known from animal experiments that the movements of the cupula correspond to those of a damped pendulum (van Egmond *et al* 1949 van Egmond, 1952)

The broken line in Fig 4 represents the times of return which would have been obtained at strengths of stimulus of 4 and 8 /sec² if the values for 1 and 2 /sec² had applied to a damped pendulum. It is seen that the unbroken line—showing our experimental results—by no means follows the broken line This implies that, at the higher strengths of stimulus, the after-discharge is shorter than the time of return of the pendulum. This is possibly to be ascribed to central nervous inhibition, which is more marked on stronger stimuli. In reality our after-discharges recorded at 4 and 8 /sec² correspond to the return times of the aforementioned pendulum only if it had been accelerated at 3.2 and 3.8 /sec² respectively

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22 klinisch gesunde Patienten wurden mit einer Beschleunigungsstärke von 1 2, 4 und 8 /sec² rotatorisch akzeleriert. Nach jeder Beschleunigung wurde die Dauer des postakz leratorischen" Nystagmus, Nachentladung genannt, gemessen. Die Nachentladung zeigte eine deutliche Tendenz, länger zu sein, je stärkere Beschleunigung a gewendet wurde, ein erhältnismäsig kleine Zunahme wurd jedoch im Beschleunigungsbereich von 4–8 /sec² notiert. Im Durchschnitt ist die Nachent ladung ungefähr die gleiche bei Stimulation in beiden Richtungen, und wenn eine Differenz vorliegt, ist diese im Durchschnitt grösser bei schwächeren Stimuli, aber es bestehen grosse individuelle Variationen und man hat für diese daher Grenz werte berechnet. Die Untersuchung hat auch gezeigt, das die Resultate nicht über einstimmen mit denen, die man bei der Bewegung eines gedämpften Pendels fi det. Bei stärkeren Stimuli erhält man nämlich eine kürzere Nachentladung, als man in solchem Falle erwartet hätte.

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TYPES OF NEURONAL ACTIVITY IN THE MEDIAL VESTIBULAR NUCLEUS

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A study of the responses of 128 neurons in the medial vestibular nucleus to ipsilateral and contralateral cold caloric stimulation revealed that 70% of those found responded to stimulation of both ears, which indicates that there is considerable crossed representation in the medial vestibular nucleus. Approximately two-thirds of the binaurally responsive neurons were reciprocally active, which suggests the influence of a crossed inhibitory arc between the two sides of the vestibular system. Other neurons in the medial vestibular nucleus appear to have representation from one side only of the vestibular system, and others appear to be nonvestibular in function.

We know considerably more about the structure and function of the semicircular ducts and the contained cupula-endolymph system than we do about the information-processing functions of the vestibular nuclei. Since Adrian's classic work (1943) *Discharges from Vestibular Receptors in the Cat* the neurophysiology of the vestibular nuclei has suffered no dramatic relevations, and the gain in knowledge in this area has been slow and tedious. The ultimate goal of neurophysiologic investigations in the vestibular nuclei would seem to be an improved understanding of the central nervous system, into which the vestibular system is highly integrated and widely dispersed, as well as a more specific understanding of the mediation of the effects of vestibular overstimulation. The study reported herein was concerned with the effect of caloric stimulation of the semicircular ducts on the activity of single neurons in the medial vestibular nucleus.

METHOD

Forty-five adult cats weighing 2.5 to 3.0 kg were used in this study. Under continuous ether anesthesia, each cat was rendered *encéphale isolé*. The animal was then fixed in a stereotaxic apparatus, after bearing points

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and the edges of the operative wound had been anesthetized with Xylocaine The occipital poles of the cerebrum and the anterosuperior part of the cerebellum were exposed through a 20-mm parieto-occipital trephine and the posterior two-thirds of the bony tentorium was removed. A microelectrode was passed vertically downward through the intact cerebellum into the brain stem near the ponto-medullary junction We entered the medial vestibular nucleus stereotaxically using coordinates from Snider's stereotaxic atlas (Snider & Niemer 1961) but depending heavily on Brodal's anatomic studies of the vestibular nuclei (Brodal *et al.*, 1962) When single neural units were found, the action potentials were recorded. The frequency of the action potentials, during the resting state and during and after stimulation, was the parameter of interest in this study

Stimulation of each labyrinth was accomplished by the delivery of 10 ml of ice water over a period of about 10 seconds into the external auditory meatus, through a canal drilled in the ear bar of the stereotaxic device Within 7 to 10 seconds after the onset of the irrigation there was an obvious change in the frequency of firing of the neural unit under investigation, if that unit was to respond at all A waiting time of at least 15 minutes between irrigations was observed. The responses of from two to eight neurons were studied in each animal At the site of the last studied neuron in each animal, a small electrolytic lesion was made, and the brain stem was removed for serial sectioning and staining (Kluver method) The locations of the neurons studied but not marked electrolytically were determined by using the recorded coordinates of the locations of those neurons.

The microelectrodes were fabricated from 200-μ tungsten wire, sharpened electrolytically until the tips were 1 0 to 2.0 μ in diameter and insulated with either *Isonel* 31 or Insl X. Electrode impedance ranged between 0.5 to 2.0 megohms.

The action potentials were amplified 2000 times, and recorded on one channel of an instrumentation tape recorder By playing the tape back into a direct writer and manually counting the spikes on the recording paper *frequency histograms were plotted for the responses of each neuron to each* stimulation When the spike frequency was relatively high and the signal to-noise ratio permitted an electronic counter was used to count the spikes.

RESULTS

The effects of ipsilateral and contralateral cold caloric stimulation on 128 units in the medial vestibular nucleus were studied. As shown in Fig 1 the change in spike frequency caused by caloric stimulation began to occur *during the irrigation usually with a brisk rise or fall,* after which the frequency of firing gradually returned toward the resting frequency One hundred twenty two of the 128 neurons (95%) exhibited either increased or decreased activity in response to caloric stimulation Approximately 80% of the neurons returned to their resting discharge rates within 150 seconds

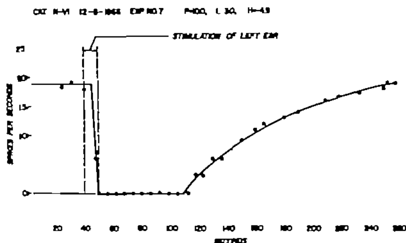


Fig. 1. Neural unit in left medial vestibular nucleus, demonstrating inhibition of resting discharge on ipsilateral stimulation, followed by post stimulatory resumption of resting discharge frequency. This unit responded in opposite manner to ipsilateral stimulation. In our classification, this is type IA neuron.

from the end of the stimulation the remaining 20% appeared to stabilize at discharge rates above or below their original resting frequencies.

The 128 units studied were classified according to their responses to cold caloric stimulation of the ipsilateral and contralateral ears. Fig. 2 shows the organization of the classification, and the percentage of units found in the medial vestibular nucleus that fall into the various categories of the classification. This classification is modeled after that of Duensing & Schaefer (1959) who, using angular accelerations, classified vestibular neurons according to their responses to ampullopetal and ampullofugal flow of endolymph. It is evident that three possible conditions can result from stimulation—an increase, a decrease, or no change in frequency of action potentials both Duensing & Schaefer's and our own classification are based on that observation.

The major significance of this study is related to the fact that the majority of the neurons which we studied were responsive to stimulation of both ears. These binaurally responsive neurons were types IA IIA, III, and IV and were 89 in number—70% of 128 neurons. Of these, approximately two-thirds were reciprocally active neurons, i.e. stimulation of one ear produced activity opposite to that of stimulation of the other ear. Of minor significance is the fact that we have added a type V neuron to the classification of Duensing & Schaefer. In this type there is no change upon stimulation of either ear.

DISCUSSION

Although Duensing & Schaefer developed their classification on the basis of acceleratory stimulation, we have chosen to utilize caloric stimulation and

and the edges of the operative wound had been anesthetized with Xylocaine The occipital poles of the cerebrum and the anterosuperior part of the cerebellum were exposed through a 20-mm parieto-occipital trephine and the posterior two-thirds of the bony tentorium was removed. A microelectrode was passed vertically downward through the intact cerebellum into the brain stem near the ponto-medullary junction. We entered the medial vestibular nucleus stereotaxically using coordinates from Snider's stereotaxic atlas (Snider & Niemer 1961) but depending heavily on Brodal's anatomic studies of the vestibular nuclei (Brodal *et al.*, 1962) When single neural units were found, the action potentials were recorded. The frequency of the action potentials, during the resting state and during and after stimulation, was the parameter of interest in this study

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covered toward the end of the experiment that hot water stimulation of either ear or merely pinching an ear elicited the same increase in activity from the type IV cell. For that reason we suspect the type IV cell to be non vestibular in function, probably related to the reticular activating system. Duensing & Schaefer (1957) suggest that both type III and type IV neurons are functionally related to the reticular activating system. Finally the type V neurons may represent projections onto the medial vestibular nucleus from unstimulated semicircular ducts, from the otolith organs, or from some nonvestibular system.

ZUSAMMENFASSUNG

In der vorliegenden Untersuchung der Einwirkung von ipsilateraler und kontralateraler thermischer Reizung mit Eiswasser auf die Aktionspotentialfrequenz von insgesamt 128 Neuronen im medialen Vestibularnukleus konnte gezeigt werden, dass 70% der Neuronen auf die Reizung sowohl des rechten wie des linken Ohres ansprachen. Es ist daraus zu entnehmen, dass eine beträchtliche Kreuzaktivierung im medialen Vestibularnukleus vorliegt. Ungefähr zwei Drittel der binaural ansprechenden Neuronen zeigten dabei ein gegensätzliches Verhalten in der Erregbarkeit bei Reizung des einen oder des anderen Ohres, was auf die Einwirkung einer Kreuzhemmung zwischen den beiden Seiten des Vestibularsystem schliessen lässt. Andere Neuronen im Vestibularnukleus scheinen nur auf Reizung einer Seite des Vestibularsystems anzusprechen, während schliesslich ein restlicher Teil der Neuronen keine vestibulare Funktion zu haben scheint.

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NON-CHROMAFFIN PARAGANGLIOMAS

Brief Review and Clinical radiological Follow-up on nine Cases

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Non-chromaffin paragangliomas, their source of origin histology symptoms and signs are briefly reviewed. Nine cases are submitted, all women aged 41–79 years. Eight patients were requested to appear for a follow-up examination immediately before the analysis. Five cases were confirmed histologically and in four the diagnosis was based upon the clinical findings. In three cases the tumour presumably arose in paraganglia in the tympanic cavity in five cases from the glomus jugulare and in one case from the carotid glomus. Three patients were not treated. In one case the diagnosis was not made until just before the present analysis. Two of the untreated patients were seen 2 and 10 years after the diagnosis had been made and showed no signs of progression One patient was treated surgically X-rays one year after the operation gave rise to a suspicion of an expanding tumour Five patients received high-voltage irradiation Two showed partial remission after a follow-up period of 5 years and three showed no signs of progression after follow-up periods of 18 months, 3 / years, and 0 years. This speaks in favour of irradiation. The radiological changes of non-chromaffin paragangliomas are briefly reviewed. The tomographic findings in eight of the patients are described Four of the five irradiated patients had tomography before and after the treatment, and the appearances are unchanged after follow-up periods of 1 and 5 years.

In close relation to the parasympathetic system there are several para ganglia consisting of non-chromaffin tissue The most important ones, connected with the glossopharyngeal and vagus nerves, are the glomus jugulare and the glomus caroticum. Marchand (1891) was the first to report a case of tumour in the carotid glomus. Rosenwasser (1945) described a case of a glomus jugulare tumour situated at the floor of the tympanic cavity

Paragangliomas of this type may have their origin not only in the glomus jugulare and carotid glomus themselves, but also in other paraganglia which may be situated in the middle ear along the auricular branch of the vagus nerve (Arnold s nerve) and the tympanic branch of the glossopharyngeal nerve (Jacobson s nerve) Tumours of the same type have been found along the aorta, in the orbit nasal cavity pancreas, and in other sites.

Several designations have been applied to these tumours, referring to their site (glomus caroticum tumour, glomus jugulare tumour, glomus tympanicum tumour). Mulligan (1950) suggested the term chemodectoma because of the presumed function of the tumour as a chemoreceptor. The term used in this paper, non-chromaffin paraganglioma (NCP), was suggested by Lattes & Waltner (1949).

NCP is a relatively rare disease. Reviewing the literature, Steinberg & Holtz (1965) found that in 1956 172 cases of tumours issuing from the glomus jugulare were on record. In Rikshospitalet, Oslo, Berdal *et al.* (1964) had found 21 cases during the period 1948–1963. The disease appears to be more common in women than in men. Out of 21 patients of Berdal *et al.* (1964) 14 were females. Grubb & Lampe (1965) reported on 20 patients, 15 of whom were women, and Lederer *et al.* (1958) on 23 patients, 15 of whom were women. In general, the disease occurs in middle aged persons, and in most cases it is unilateral. However, Resler *et al.* (1966) found several accounts of multiple tumours in the literature. Possibly there is an hereditary predisposition.

Histological examination shows round or polygonal cells with faintly eosinophilic granules and a round or oval nucleus of variable size with small, frequently multiple nucleoli. The cells are arranged in clusters in a highly vascular stroma of collagen fibrils with capillaries and sinusoids of varying size and numerous arterio-venous anastomoses throughout. Malignant changes have been described by, amongst others, Berdal *et al.* (1964). Reese *et al.* (1963) reviewed the literature on the tendency to malignancy in tumours issuing from the carotid glomus and added a case of their own, the thirteenth to be reported of lymph node metastases. NCP in other sites have been known to give rise to local lymph node metastases as well as to distant metastases. In general, however, the tumour is histologically benign, but with a tendency to local recurrence.

The non-chromaffin paraganglia act as chemoreceptors, sensitive to the oxygen tension in the arterial blood. A fall in this tension elicits increased ventilation, an accelerated heart rate, and possibly an elevation of blood pressure. The chemoreceptors may also be sensitive to changes in the carbon dioxide tension and in pH. Adrenaline production has not been demonstrated, while noradrenaline has been found by histochemical methods. It cannot be ruled out that tumours in these paraganglia may cause hypertension in youngish individuals, as pointed out by Steinberg & Holtz (1965).

Tumours arising in the glomus caroticum, glomus jugulare and other similar paraganglia, then, are histologically identical, but yet it is practical, for symptomatological reasons, to classify them topographically into (1) non-chromaffin paragangliomas arising in the glomus caroticum, (2) non-chromaffin paragangliomas in the middle ear and (3) non-chromaffin paragangliomas arising in the glomus jugulare, as suggested by Capps (1958) and by Lederer *et al.* (1958).

These tumours are slow-growing, and symptoms are due to local expan

sion Frequently they are not diagnosed until a late stage In Steinberg & Holtz s case symptoms had been present for 50 years, in Berdal *et al* s (1964) series there are cases with a 10–12 year history and Schmidt (1967) has published a case of a very small tumour behind the tympanic membrane with a 10-year history

Symptoms and Signs

Glomus caroticum tumours are usually painless. The symptoms and signs are due to their pressure upon the surrounding structures, resulting in hoarseness, cough, dysphagia, nausea, headache, and tinnitus. The tumour is palpable on the neck, below the angle of the jaw anterior to the sternocleidomastoid muscle movable in the horizontal plane but not in the vertical plane The external carotid artery may be palpable, stretched over the lateral aspect of the tumour Some tumours grow inwards, presenting a smooth, ovoid mass on the lateral pharyngeal wall, in some cases with medial displacement of the tonsil.

NCP arising in the glomus jugulare and paraganglia in the middle ear usually manifest themselves first in unilateral hearing loss, followed by pulsating tinnitus. There may be aural discharge, and at a late stage spontaneous haemorrhages In addition, vertigo, nausea, and pain deep in the ear Facial palsy has been observed in a number of the cases, and the 9th, 10th 11th, and 12th cranial nerves may be affected, leading to dysphagia, paresis of the vocal cords, and atrophy and paresis of the tongue Horner s syndrome has been reported, indicating affection of the sympathetic trunk Otoscopy reveals a reddish mass in the auditory meatus or in many cases behind an intact drum usually arising infero-posteriorly The tumour may be pulsating and compressible by the use of Siegle s otoscope and paracentesis will cause profuse haemorrhage X-ray examination of the temporal bone supplemented by tomography may show erosion of the petrous portion, and there may be destruction of the base of the skull around the foramen jugulare Gejrot (1964) and Hamberger & Gejrot (1964) demonstrated the diagnostic significance of retrograde jugularography especially in cases where the tumour arises in the jugular bulb

In the differential diagnosis the carotid type belongs to the large group of other neck tumours, such as branchial cysts, nodal enlargement due to infection or systemic diseases, metastases, neurofibromas, aneurysms, etc. Where the NCP is situated in the middle ear and in relation to the jugular bulb, the differential diagnostic possibilities are chronic polypous otitis media, benign granulomas in the external auditory meatus, carcinoma or sarcoma in the middle ear angiomatous tumours, malformation of the jugular bulb, neurinomas arising in the 5th–12th cranial nerves, or primary tumours in the base of the skull The diagnosis is made by biopsy but heavy bleeding may occur

Treatment

The treatment of NCP has been a matter of discussion, the conflict being between radiotherapy and surgery. Berdal et al. (1964) had 17 cases of NCP in relation to the temporal bone. All were treated surgically with radical excision in 12 cases, and only one patient received supplementary irradiation. Out of four tumours of the carotid type, three were excised and one irradiated. Thus, Berdal et al. prefer surgical treatment in all cases of NCP. Capps (1953) recommended radiotherapy of tumours which arise in the glomus jugulare, as they are often large and of difficult access. He also felt that recurrences following excision ought to be irradiated. The same view was expressed by Lederer et al. (1958) who tried radical operation on all minor tumours in the middle ear followed by postoperative irradiation. Williams (1955) found that the growth of the tumour could be arrested by radiotherapy which he recommended, possibly followed by operation. Brown (1953) treated six cases of tumours in the middle ear by operation and postoperative radiation. On the basis of a series of 20 patients Grubb & Lampe (1965) earnestly recommended radiotherapy alone, or postoperatively if small tumours were treated surgically. These authors do not feel in a position to secure a complete destruction of these tumours, recommending observation until the effect of the treatment can be assessed. Farrior (1967) emphasized that small NCP in the middle ear ought to be removed radically without any follow up and reported five operated cases with preserved hearing. None was irradiated.

Thus, as also emphasized by Steinberg & Holtz (1965), there seems to be a tendency to administer radiotherapy under all circumstances, possibly after partial or radical removal of the tumour. Most authors feel that positive results are obtained by radiotherapy.

Present Material

During the period 1958–1967 a total of nine cases of NCP were diagnosed in the University Department of Otolaryngology, Rigshospitalet, Blegdamsvej, Copenhagen. In five cases (Nos. 2, 3, 4, 6, and 9, Table 1) the diagnosis was histologically confirmed, and in the remaining four cases it was based upon the clinical and radiographic findings. All the patients were women, aged 41–79 years (average age 58 years). The study is retrospective. All the patients were requested to appear for a follow-up examination immediately before the analysis.

Clinical Findings (Table 1)

Judging by the symptoms and signs, the tumour had arisen in three cases (Nos. 4, 6, and 7) in paraganglia in the tympanic cavity and was not accompanied by neurological symptoms. In five cases it had presumably

Table 1

Case Age Sex	Lengt of history years	Symptoms	Otologic signs	Cranial nerves i volved	R diography of temporal bone	Radiotherapy	Verified by hi tological examination	Surgical procedure	Course
1 11 ♀	2	Tinnit headache	Objective tinnitus	VII	Destruction of jug. foramen, deformed j gular b lb	–	–	–	–
2 16 ♀	8	Impaired hearing, pain of the ear tinnitus, hoarseness	Pulsating drum, impaired hearing	VIII X	Destruction of j g foramen	+ 6000r/7 weeks	+	Explorative tympanot my	N sign f progression 6 yrs
3 53	10	Impaired hearing, difficulty in sw llowing, hoarseness	Big polypus f meatus, anacusis, spontaneous ystag mus, tumor of the nasal pharynx nd tonsillar region	VII VIII X, XI XII	Destruction f base of sk ll jug. f ramen and temporal bone	+ 1130r/3½ weeks	+	Explorative proced re on the eck with biopsy	R mission See t t
4 57 ♀	6	Impaired hearing	Red tumor bulging the drum, impaired hearing	VIII	N osseous destruction	–	+	R dical ma toidect my Removal of t mor from middle ear	S spicion of t mor in the j g. foramen on radiography one yea aft r operation
5 67 ♂	10	Impaired hearing, tinnitus	Pulsating tumor behind the drum impaired hearing, vestibular hypof nc-tion, t mor of the nasal pharyn	VII (Impaired tast) VIII	N osseous destruction	+ 6000r/6 week	–	–	N sign f progression 3½ yrs

6 79 ♀	1	Impaired hearing, otitis	Big tumor in meatus, impaired hearing	VIII	No osseous destruction		+		No sign of progression 2 yrs
7 71 ♀	2	Impaired hearing, vertigo	Tumor bulging the drum, impaired hearing, vestibular hypofunction	VIII	No osseous destruction	+ 6500r/6 weeks		–	No sign of progression 1½ yrs
8 53 ♀	3	Impaired hearing, hoarseness	Small tumor behind the drum	VIII, X, XII	Expansion of jug. foramen	+ 1000r/5 week	–	–	Remission See text
9 60 ♀	10	No symptoms	Lump on the left side of the neck				+	Explorative procedure with biopsy	No sign of progression 10 yrs

arisen in the glomus jugulare in close relation to the hypoglossal nerve as well as the 9th, 10th and 11th cranial nerves in the foramen jugulare. In one case (No. 9) the tumour had originated in the carotid glomus.

Since in four cases (Nos. 2, 3, 5 and 8) the tumour also manifested itself in the tympanic cavity it cannot be excluded that in some of these cases too it had arisen in the middle ear and later progressed to the foramen jugulare. On comparison of the clinical and the radiological criteria, the classification of the cases into tympanic and jugular types proved to be congruent. In one case (No. 8) otoscopy showed a small tumour in the tympanic cavity which had not been diagnosed by X rays.

The tumours had given rise to symptoms for an average period of 6 years from 2 to 10 years) before the patients were first seen.

All but one of the patients with tympanic or jugular tumours complained of homolateral pulsating tinnitus. Only one patient complained of dizziness (homolateral canal paresis). Three complained of hoarseness (all had paralysis of the homolateral recurrent laryngeal nerve).

In seven patients physical examination showed a tumour in the auditory meatus or (more often) behind the drum. Pulsation of the tumour was found in only two cases. Two patients had normal hearing, five had homolateral, severe conduction loss, accompanied by less marked perceptive hearing impairment. One patient had homolateral anacusis. Differential caloric tests had been done on four patients, two of whom had homolateral canal paresis.

Five patients had neurological symptoms or signs which could be referred to one or more of the 7th, 9th, 10th, 11th, or 12th cranial nerves.

The patient with a carotid glomus tumour had no symptoms. Physical examination showed a tumour 3 × 8 × 8 cm, on the left side of the neck, but no other abnormalities.

Treatment and Course (Table 1)

Three patients had received no treatment. Five had received high voltage irradiation (from 4000 to 6500 r).

In one patient (case 4) the tumour was removed surgically. Operation showed the tumour to be apparently restricted to the antrum and tympanic cavity. The incus and malleus were embedded in tumour tissue and had to be removed together with the drum.

Out of the three untreated patients one (case 1) had the tumour diagnosed immediately before the present analysis and has not yet been seen for a follow up examination. Case 6 (in whom the diagnosis was confirmed histologically) had a history of symptoms for 4 years and had been followed for another 2 years without showing clinical or radiographic signs of progression. Case 9 (also confirmed histologically) when examined 10 years after the diagnosis had been made showed no signs of progression.

The 5 patients who were treated by radiotherapy were examined 18 months – 6 years (average 4 years) after the treatment was completed. In

two of the patients, whose tumours had caused the most severe symptoms (cases 2 and 8) the radiotherapy resulted in partial or complete remission

Case 3 (histologically confirmed) showed, prior to the treatment, a large tumour filling the entire auditory meatus, a large swelling behind the tonsil, a tumour visible in the rhinopharynx, partial paresis of the 7th and 12th cranial nerves, total paresis of the 10th and 11th. Eight months after the treatment, the tumour in the auditory meatus had subsided to "questionable tumour tissue at the floor of the auditory meatus" the retrotonsillar swelling had considerably diminished, and a tumour was no longer visible in the rhinopharynx. The facial palsy had remitted, while the other pareses persisted. These findings were unchanged 5 years after the completion of treatment Straight X rays revealed extensive destruction of the bone around the foramen jugulare, involving the lateral border of the foramen magnum and the middle ear Tomography of the base of the skull (Fig 1) and temporal bone showed that the massive destruction of bone around the foramen jugulare extended into the foramen magnum, involving the canal of the hypoglossal nerve, destroying anteriorly the posterior surface of the petrous portion and the internal auditory meatus, and involving superolaterally the tympanic cavity vestibule, cochlea, and ossicles (Fig 2) The posterior wall and floor of the external auditory meatus were also eroded These findings were unchanged 5 years after radiotherapy

Case 8 showed, prior to the treatment, a visible tumour behind the drum, paresis of the soft palate and of the recurrent laryngeal nerve, and paracenteals yielded "a flood of blood" Two months after the treatment the tumour was no longer visible behind the drum, and paracenteals did not yield blood. The soft palate paresis had remitted but that of the recurrent laryngeal nerve persisted. These findings were unchanged 5 years after the completion of treatment. Tomography was required to reveal, with certainty bone destruction of the foramen jugulare, involving the canal of the hypoglossal nerve and the lateral border of the clivus (Fig. 3) In addition, there was erosion of the apex of the petrous portion and of the floor of the internal auditory meatus. Five years after the irradiation the findings were unchanged.

In the remaining three patients radiotherapy had afforded remission for only a few months. Thereafter the condition returned to a stage which clinically corresponded to the pre-treatment condition. At follow-up 18 months, 3½ years, and 6 years after the treatment, however there had not been further progression.

Radiology

The radiological diagnosis in the present material was based upon X rays of the temporal bones by the conventional technique and upon tomography of the temporal bones as well as the base of the skull The Philips-Massiot polytome with hypocycloid movement was used in all cases. The AP projec





Fig 1 A case f hist logically confirmed gl mu t mou (case 3) cli ically l ibl i the left external ditory meatu nd i the rhinopharynx. Total paresis f the 10th nd 11th, partial paresi of the 7th and 12th cranial nerv Tomography in the speci l f ramen jugulare projecti n show ma si bone destructi n aro nd the f ramen jugulare n the left side extendi g int th f ramen magnum. Also, bone d structi i learly seen t the posterio surface f the petro porti n, th tympa i cavit ossi les d the estibule Th sseou w ll between the hypot mpa and the f ramen j gul re i destroyed complet ly

Nomenclature

2 Tympani cavit
2 Epitympa ic recess
2 Hypot mpani reces
3 Audit ry ossicles
3 M ll
4 M st id ntrum
5 M st id process
8 Cochlea
8 Cochlea, basal t rn
9 Vestib l f the lab rinth
10 Lateral sem circul canal
11 Superio semicircul canal
13 Internal acoustic meat
19 Jugula f ssa (bulb f j gular ei)
*6 H poglossal ca l

Rad Op. Defect of radical operation
(alpha) = Path logical ha ge (g. deformed ossicles etc.)





Fig 2. Same patient f Fig 1 (case 3) but the t mogram lies nterio t th preceding Here the bone destructio nvol es also the basal coil f the cochlea nd the canal f the hypoglossal nerve





Fig 3. Case 8 Tomogram i the special foramen j gul re projection showing wideni g and bone destruction of the foramen jugulare, i ol ing the canal f the hypoglossal nerv I thi case destruction i co fined t the f ramen j gulare The middl nd i ner ea is undamaged, especially i no ero ion f the floor f the tympani ca ity t be observed

tion was used for visualizing the petrous portion, in one case supplemented by an axial-pyramidal projection. To visualize the base of the skull around the foramen jugulare, the subject was positioned with head extended 45 in relation to the horizontal plane. This makes the plane of the foramen jugulare parallel with the film i.e the foramen is visualized *en face*

For technical data (cf Rovsing & Jensen 1968)

Out of the nine patients seven were examined tomographically in the AP projection, while seven also had tomography in the special foramen jugulare projection Four out of the five irradiated patients had tomography before and after the treatment, the fifth one only after The interval between the radiotherapy and the last X ray examination ranged from 1 to 5 years.

The radiological diagnosis was based upon soft tissue and skeletal changes which do not differ in nature from other lesions in this region, but which give rise to a suspicion of glomus tumour by virtue of their site and extent (Frey 1967) The changes consist in blurring of the soft tissues and/or bone destruction. The blurring of the soft tissues occurs in the tympanic cavity the epitympanic recess, and hypotympanon but may also present itself in the foramen jugulare. The skeletal changes are osteolytic and may involve the entire middle and inner ear destroying the apex of the pyramid, the third segment of the facial canal, the osseous border of the foramen jugulare the mastoid process, the hypoglossal canal clivus, sphenoid bone with the sphenoid sinus, and the mandibular joint (Kemp Harper 1957 Frey 1967)

According to the site, the tumour may be said to be of the jugular or tympanic type. The former manifests itself on the tomograms as bone destruction around the foramen jugulare the posterior and inferior wall of the petrous portion and occipital bone while the middle ear is normal In the tympanic type there is a soft-tissue shadow in the hypotympanon, tympanic cavity and antrum, bone destruction in the lateral antral wall and destruction of the ossicles More advanced cases show destruction of the thin bony wall between the foramen jugulare and tympanic cavity In addition, mixed types are often seen, especially at later stages of the disease with extensive changes in the temporal bone as well as the base of the skull

The present material included three cases (Nos. 4 6, and 7) of the tympanic type with soft-tissue shadows in the middle ear but no bone destruction. In these cases the foramen jugulare was of normal appearance

Two cases (Nos. 1 and 8) belong to the jugular type showing severe bone destruction around the foramen jugulare in both cases involving the posterior wall of the petrous portion, the apex and floor of the internal acoustic meatus, while the middle ear was completely intact Case 1 also had retrograde jugularography but this will be reported separately

The remaining 3 cases (Nos. 2, 3, and 5) are mixed types. One exhibited soft-tissue shadows in the foramen jugulare and tympanic cavity but no bone destruction. Another one had bone destruction around the foramen jugulare and of the wall against the middle ear Case 3 cf case history

The two cases of jugular changes also exhibited partial or complete erosion of the hypoglossal canal, while the facial canal was destroyed only in the one case mentioned above

Dilatation of the foramen transversarium atlantis on the affected side, as demonstrated by amongst others, Kemp Harper (1957) due to dilatation of the vertebral artery caused by increased vascularity in the base of the skull was not observed in the present series. The explanation is perhaps that the projections used do not properly visualize the atlas.

Comparison of pre-treatment X rays with X rays obtained after a follow up period of 1–5 years showed in four of the five irradiated cases unchanged appearances. Thus, the bone changes had not regressed. This is in conformity with the reports of others (Kemp Harper 1957) On the other hand, tomography has not demonstrated any progression of the skeletal changes after the radiotherapy

The only surgically treated, non irradiated patient (case 4) showed bone destruction of the foramen jugulare one year after the operation.

DISCUSSION

As already mentioned, the non-chromaffin paraganglioma is a fairly uncommon disease We have come across nine cases in 10 years. From Rikshospitalet, Oslo, 21 cases have been reported in 15 years. All published materials have shown a definite female preponderance. All the patients of the present series were women. It is stated that the disease is most often diagnosed in middle-aged persons, and this fits with the age distribution of the present patients (41–79 years)

According to the literature, 5–10 years generally pass before the disease is diagnosed. This is due partly to the slow growth of the tumour and partly to the fact that in spite of all the disease is so rare that signs such as a large "polyp" in the auditory meatus or paresis of the recurrent laryngeal or of the hypoglossal nerve do not make one think of non-chromaffin paraganglioma.

In our material too an average of 6 years elapsed before the disease was diagnosed. This long period is due, however primarily to the patients delay in seeking medical advice as they were not particularly bothered by the symptoms. After the patients were first seen, the diagnosis was made in the course of a short time—in five cases at otoscopy possibly supplemented by paracentesis which yielded profuse bleeding In three of the patients (cases 1 3, and 8) X ray examination supported the clinical suspicion of a glomus tumour

In cases 2, 3, 4, 6, and 9 the diagnosis was confirmed histologically This was not done in cases 5 7 and 8 but the diagnosis must be considered as definite as is possible without biopsy (pulsating tumour behind the drum, profuse bleeding on paracentesis, temporary remission following radiotherapy a history of 3½ 8, and 13 years) Only case 1 did not exhibit a

tumour on otoscopy and this patient had neurological signs only from the 12th cranial nerve Tomography of the temporal bones and retrograde jugulography of the left internal jugular vein, however afforded strong indications of a glomus jugulare tumour

The therapeutic results in this small series speak in favour of radiotherapy Two patients (cases 3 and 8) have obtained complete or partial remission of the tumour without any signs of recurrence at the end of 5 years. Incidentally it was in these two patients that the tumour had given rise to the most severe symptoms and signs, clinically as well as radiologically In both, the manifest cranial nerve pareses were unchanged after the treatment while milder cranial nerve pareses disappeared In the other three irradiated patients the tumour remitted only temporarily but on the other hand there have not been further signs of progression after follow up periods of 18 months, 3½ years, and 6 years It may be added that the tumours have also not progressed in cases 6 and 9 who have been followed untreated for 2 and 9 years respectively without radiotherapy or surgical treatment

In the two patients whose tumours yielded to radiotherapy neither the total dose nor the dose per time unit had been larger than in the other three patients

The radiotherapy did not cause hearing impairment or other permanent damage in any of the five patients (one had anacusis before the treatment)

The only patient (case 4) who was treated surgically developed a severe aggravation of her hearing impairment as the malleus and incus had to be removed In addition, X ray examination one year after the operation gave rise to a suspicion that now there is an expanding tumour in the foramen jugulare

ZUSAMMENFASSUNG

Es wird über nichtchromaffine Paragangliome deren Ausgangspunkte Histologie und Symptomatologie eine kurze Übersicht gegeben. Neun Fälle werden vorgelegt alle Frauen im Alter von 41 bis 79 Jahren. 8 Patienten wurden unmittelbar vor der Aufstellung zur Nachuntersuchung aufgerufen. 3 Fälle sind histologisch verifiziert, in 4 Fällen stellte man die Diagnose klinisch fest In 3 Fällen geht der Tumor vermutlich von Paraganglien im Cavum tympani aus in 5 Fällen vom Gl mu jugulare und in einem Fall vom Glomus caroticum. 3 Patienten wurden nicht behandelt In einem Fall wurde die Diagnose erst kurz vor Aufstellung dieser Arbeit gestellt Bei 2 Fällen sind die Patienten 2 und 10 Jahre nach der Stellung der Diagnose n chuntersucht worden dabei wurde eine Tumorprogression nicht beobachtet 1 Patient wurde chirurgisch behandelt Eine Röntgenuntersuchung 1 Jahr nach der Operation gibt Verdacht auf Tumorwachstum Patienten wurden hochvoltbestrahlt. In 2 Fällen kommt bei einer 5jährigen Beobachtungszeit eine teilweise Remi sion vor und in 3 Fällen erscheinen keine Anzeichen einer Progression nach 1 , 3 , und 6jähriger Beobachtungszeit. Die radiologischen Änderungen bei nichtchromaffinen Parag ngliomen werden kurz durchgearbeitet 8 Fälle wurden tomographi ch untersucht und der Befund beschrieben 4 von 5 Fällen,

die mit Strahlen behandelt wurden, wurden vor und nach der Behandlung tomographiert und zeigten nach einer Beobachtungszeit von 1 bis 5 Jahren röntgenologisch keine Veränderungen auf.

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THE EFFECT OF SURGICALLY INDUCED RECURRENT LARYNGEAL NERVE PALSIES ON THE LARYNX OF THE DOG AND SUBSEQUENT MODIFICATION BY SUPERIOR LARYNGEAL NERVE SECTION

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In fourteen dogs measurements of the width of the rima glottidis were made after cutting the left recurrent laryngeal nerve and after subsequent section of the external ramus of the ipsilateral superior laryngeal nerve. *Direct measurements using dividers and indirect measurements from a* cine-film were compared and showed an increase in the width of the rima glottidis in thirteen of the dogs using one or other method of measurement. However in three of the dogs one method of measurement failed to show an increase. The rima was widened in one dog after an interval of four months between the two operations. A much smaller series using dogs with bilateral recurrent laryngeal nerve palsies was inconclusive. The possibility of applying a similar technique to inactivate the crico-thyroid muscles in recurrent laryngeal nerve palsies in the human is discussed.

The release of the vocal cords from the adducted position in recurrent laryngeal nerve paralysis by section of the branch of the superior laryngeal nerve supplying the cricothyroid muscle has been described before. Some of the results were of clinical benefit to patients (Martens, 1911) and have been reviewed by Tschiassny (1957) while others were experimental (Lemere 1933 Fischer 1952). No series of adequate length on this subject however seems to have been reported with a quantitative assessment of the changes brought about. If paralysis of the cricothyroid muscle were shown to offer the prospect of relieving the adducted position of the cords in bilateral recurrent laryngeal nerve palsy in humans, it might present a practicable alternative to tracheostomy in acute cases or to arytenoidectomy or similar procedures in long-standing cases. A quantitative experimental study was therefore undertaken to determine the degree of opening of the glottis in recurrent palsy after paralysis of the cricothyroid and to assess whether there is a period of time after which the larynx cannot be modified owing to degeneration of the joints and ligaments and/or to atrophy of the crico-thyroid muscle it is intended to paralyse.

Dogs were chosen as subjects because of their availability the prospect of correlating the results with previous work (Lemere 1933 Fischer 1952



Fig. 1. Dissection of the neck of the dog. *A* left recurrent laryngeal nerve. *B* external ramus of left superior laryngeal nerve supplying cricothyroid muscle. (*C*) (*D*) Sterno-thyroid muscle. (*E*) Internal carotid artery; (*F*) Trachea.

Mårtensson, 1963 and 1964) the ease with which the larynx may be directly viewed and the rough similarity in size and proportions to the human larynx.

MATERIALS AND METHOD

The dogs were anaesthetized with intravenous nembutal for operative procedures and with thiopentone for merely viewing the larynx. Direct laryngoscopy with filming and measurement of the width of the glottis was helped by a few simple measures: the dog was laid on its back and a spring gag placed between upper and lower incisors. The tongue was pulled as far out as possible and the tip then strapped beneath the jaw. It only remained for the epiglottis to be held forwards by a very thin spatula for the larynx to be viewed quite easily under direct vision. The width of the glottis was measured with a pair of long dividers with screw-operating adjustment. Soon after the experiments were begun a few frames of a colour cine-film were taken at the same time.

The recurrent laryngeal nerves were exposed by a midline longitudinal incision of the neck. The strap muscles were separated in the midline and all the tissue, including the thyroid gland, was dissected from the side of the trachea until the recurrent laryngeal nerves were found. These were tied in

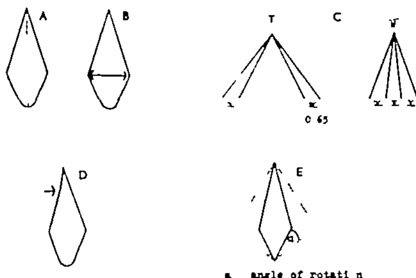


Fig 2 Diagram ill strati g the positions of the vocal cords. F r explanatio see text.

two places, usually about 2 cm caudal to the larynx, and were cut between the ligatures (Fig 1) In most cases the trunk was single but in nine dogs it was double and in one it was treble It was judged that, in the latter case, at least one of the trunks supplied the oesophagus but to be sure that the palsy should be complete every longitudinal filament was sectioned

The branch of the superior laryngeal nerve to the cricothyroid muscle (the external laryngeal nerve) was identified after retracting the sternohyoid muscle In the dog it curves medially deep to the sternothyroid at this level in the neck The cricothyroid muscle was made to twitch on tying before cutting lest the nerve should be mistaken for a branch of the ansa hypoglossi

In most of the dogs only the left recurrent nerve was cut and the glottis was observed for periods of time varying from a few minutes to 127 days. At the end of a given period the left external laryngeal nerve was cut and any further change in the glottis was noted.

The width of the rima glottidis was measured almost every week. The only sure way of assessing the success of recurrent laryngeal nerve section was to note the absence of abduction in inspiration on the affected side The width of the rima was measured during the longest period at which the cords were immobile which was from near the end of expiration until the end of the pause before inspiration. At this time the larynx appeared symmetrical, both cords being equidistant from the midline in the "intermediate" position (Figs. 2 A and 3)

Since the changes in position resulting from either operation were not expected to be great the accuracy of the measurements was all the more important particularly since in every dog the width of the glottis varied a little from week to week. The direct measurements of the distance between

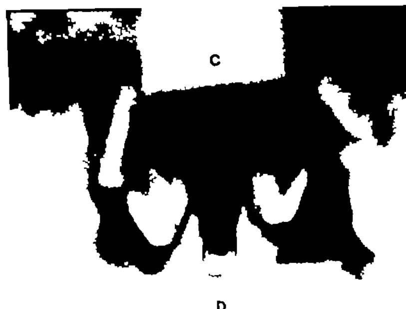


Fig 3 Direct view of larynx of dog Ventral position at end of expiration. The width of the rima glottidis was measured between the points marked (*A*) at the tips of the vocal processes of the arytenoid cartilages. (*B*) Vocal cord; (*C*) Spatula holding the epiglottis anteriorly; (*D*) Soft palate

the tips of the vocal processes of the arytenoids, i.e the widest part of the diamond-shaped glottis (Fig. 2 B) were supplemented by cine-films when possible shortly after the series started. The reason for the films, apart from the permanent record of the appearances of the larynx in various combinations of palsies, was to allow magnification of the glottis so that the measurements might be more accurate. The 16 mm films were analysed in a microfilm viewer one frame at a time Here the image was at least twice the size of the actual larynx and the same measurement was taken by placing the dividers on the screen. This allowed more time for accuracy Each time a larynx was filmed, a film was taken of the dividers exactly one centimetre apart placed the same distance away from the camera as the larynx. All readings on the enlarged film were therefore referable to a 1 cm standard

Limitations and Merits of the Method

The dogs were always under a general anaesthetic when the cords were viewed and the effect of an effort to bark or otherwise voluntarily adduct the cords could not be observed. To some extent even the reflex movements of the cords were affected by the anaesthetic. Thus, for example, the breathing of the anaesthetised animal was slow and regular and so too, of course

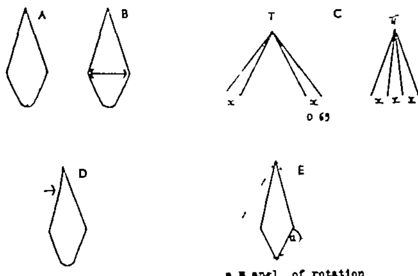


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Table 1 *The width of the rima glottidis before and after section of recurrent and external laryngeal nerves*

	Subject	Before section of left recurrent nerve. ( )	After section of left recurrent nerve (or)		After section of left external laryngeal nerve. (or s)
Direct readings	1	no reading	5.0 mm		6.5 mm
	2	6.0 mm	4.7		5.4
	3	7.5	5.9		7.2
	4	6.25	4.7		5.5
	6	4.0	3.6		4.0
	7	6.5	6.7		6.7
	9	4.5	3.8		4 1
	10	4.6	4.8		5.6
	11	6.5	6.2		6.5
	12	6.0	5.8	a	5.5
	13	8.7	7.0		7.2
	14	4.9	3.9		4.2
Film readings (later method)	2		4.76		4.79
	4		4.86		4.23
	6		3.60		4.23
	7		6.18	a	6.59
	8		4.30		4.60
	9		2.93		3.50
	10		4.58		4.50
	11	7.24	6.13		6.97
	12	6.20	5.27		5.88
	14	4.70	3.64		3.70
Film readings (early method)	1		5.08		5.18
	3		6.54		6.65
	5		5.18[x]		6.53[x]

a, verag al es.
s, *singl lues.*
x, width i abduction.

external laryngeal nerve at the second operation 1 to 8 days later Two dogs were first given bilateral recurrent laryngeal nerve palsy This was followed by a bilateral external laryngeal nerve palsy on the same day in one dog and a few days later in the other

Reference to Table 1 indicates that out of 12 dogs measured directly after the initial left recurrent nerve section the width of the glottis was reduced in nine of the dogs and was increased in two. In dog no 1 there was no pre section reading The average reduction in width in the dogs where this occcurred was 0 97 mm. Pre-section films were only taken in three of the dogs and in all of them width was reduced. Again, from direct readings after cutting the left external laryngeal nerve, the rima glottidis was widened

Table 2. *Width of rima glottidis in mm in the two dogs kept for the longest period*

Readings in chronological rder

- standard devi tion.

v = coefficient f variation.

Subject	Method of measuring	Before left recurrent nerve section	After left[#] recurrent nerve section	s	v (%)	After section f left external laryngeal nerve
2	Direct	6.0	4.5 4.25 3.7 4.6 4.8 5.4[f] 4.5[f] 4.2 4.5[f] 4 7[f] 5.3[f] 4.8[f] 5.9	0.55	11.6	6.1 5 1 4.9
2	Film		5.39 4.74 4 12 4 74 5.02 4.56	0.39	8.2	4.48 5.11
9	Direct	4.5	5.0 3.5[f] 3.0[f] 3.0[f] 3.5[f] 4.0[f] 4.5	0.70	18.4	5.0 3.8 3.7 4.0
9	Film		2.78 2.54 2.58 3.12 3.63	0.58	19.8	3.49 3.72

f film measurement taken at same time nd shown in same rder below

Increase i verage value fo the last half f this period over verage f r whole period is follows:

Subject	Method	
2	direct	0.31 mm
	film	0.01 mm
9	direct	0 10 mm
	film	0.28 mm

in 10 dogs, unchanged in one dog and diminished in one The average in crease in width in the dogs where this occurred using direct measurements, was 0 0ɔ mm. Similar results are shown in the film measurements.

Measurements were confined to the "resting" period at the end of expiration because the extent of abduction was not consistent Furthermore it was impossible to take a direct measurement during abduction because the cords were not stationary in this position The films did not show abduction often because respiration was slow and most readings were only for two or three seconds. Moreover the cords could seldom have been filmed in *maximum* abduction because generally the dogs were breathing slowly and lightly The abducted position was less constant and more subject to the depth of respira tion and to the G A than the "resting" or "neutral" position.

To obtain a rough guide to the accuracy of the direct measurements and the later film measurements the readings taken in the interval between the two operations were analysed in more detail in the two dogs where this interval was longest (Table 2) In one dog (no 2) the coefficient of variation

was 11 6% in the direct readings and 8.2% in the film readings. In the other dog (no 9) the coefficients were 18.4 % and 19.8% respectively indicating that in this dog the variations were, surprisingly greater in the film measurements.

There is a danger that a widening of the rima glottidis following section of the external ramus might have happened anyway Controls for the dogs with short time intervals between operations were provided by those with long time intervals. Where more than one measurement was made during this interval the average value for the latter half of the readings was compared with the average for all the readings in the interval in each dog to discover any tendency for the width to increase in time spontaneously Using the direct measurement it was found that in eight dogs the values in the last half were above those in the first half in seven they were less and in one they were the same In the film measurements in five dogs the width was greater in the second half and in three it was smaller These changes must be taken into account when assessing the degree of widening brought about by external ramus section alone, but they do indicate that the tendency for widening to occur spontaneously is slight.

The changes in the rima after all the operations were very small and there was therefore little margin allowable for inaccurate measurements. It was at first not expected that so little adduction of the cords would result from complete section of the recurrent nerve Indeed, in the two dogs in which bilateral recurrent section was undertaken the cords were adducted by only 0.2 mm in one and actually abducted by 0.2 mm in the other With such slight initial adduction it was not expected to obtain a great deal of abduction by cutting the ipselateral external ramus and such was not indeed the case. However taking the filmed and direct results separately and together there is good reason for thinking that the width of the glottis increases a little after ipselateral section of the external ramus in nearly all cases

Where both recurrent nerves and one external ramus were cut together initially the results of cutting the contralateral external ramus later were disappointing and inconclusive (Table 3) This series was undertaken in the hope that any action of the *remaining recurrent nerve in the previous series*, such as compensatory abduction or an action on the interarytenoid muscle affecting the cords on the cut side, would be abolished.

Apart from the absence of abduction during inspiration it was impossible to tell on which side a recurrent nerve had been cut, as the cords were roughly equidistant from the midline The dogs lay on their backs and no lack of bracing back of the arytenoid could be detected. Sometimes the paralysed vocal cord did hang inwards slightly (Fig 2 D)

The rima of the dog is diamond-shaped at all degrees of abduction and the lateral sliding component of the arytenoids seen in the human is minimal in these animals. Presumably the interarytenoid muscle is less important. The predominant movement of the arytenoids was rotation (Fig. 2 E)

It was confirmed by tetanic stimulation of the peripheral end of the

Table 3 *The width of the rima glottidis*

	Subject	Before section of both recurrents and one external laryngeal nerve	After section of both recurrents and one external laryngeal nerve		After section of the remaining external nerve	
Direct readings	15	5.7 mm	5.7 mm	*a*	5.1 mm	
	16	7.5	6.4	*a*	6.1	*s*
	17	6.6	6.15	*a*	5.5	*s*
	18	5.8	5.25		4.3	
	19	7.6	7.3	*a*	7.5	*s*
Film readings (later method)	15		4.98		5.53	*s*
	16		6.56	*s*	5.68	*s*
	17		4.78		5.79	*s*
	18		5.08	*s*	4.83	*s*
	19		6.99	*s*	7.17	*s*
	20[i]		5.42		5.68	
	21[ii]		4.41	*s*	8.33	*s*
		Before section of both recurrent laryngeal nerves	After section of both recurrent laryngeal nerves		After section of both external laryngeal nerves	
Direct readings	22[i]	7.8 mm	7.4 mm		8.3 mm	
	23	9.0	9.2	*a*	8.1	*a*
Film readings (later method)	23		8.05	*a*	7.18	*s*

a average values.
s, [s]ingl[e va]lues.
i [A]ll nerves sectioned and readings taken i[n o]ne operation.
ii, L[a]st three nerves sectioned in [o]ne operation.

severed external ramus that the cricothyroid muscle is capable of adducting the vocal cords to the midline When the animal was lightly anaesthetised and breathing heavily the cricothyroids, if exposed, could be seen contracting in time with expiration the cords would be more than usually adducted at this time and were often lengthened.

In this series, surprisingly there was evidence in only one dog of a second motor nerve supplying the cricothyroid when it was tied the muscle twitched and after it was cut the muscle continued contracting in expiration until the genuine ramus was cut On the opposite side a second nerve was again present but it supplied the sternohyoid.

DISCUSSION

In the dogs in these experiments there was not enough adduction to hinder breathing had the recurrent nerve been cut on both sides and so section of the external ramus would not have been indicated clinically and

it would not have reduced the air resistance significantly Had the cords been well adducted after recurrent nerve section, which we had expected to be the case (Lemere, 1933 Fischer 1952 Freedman, 1955) we should have expected section of the external laryngeal nerve to result in a more significant abduction as far as the intermediate (cadaveric) position.

Since this was the position in which the cords ended, they must have been very near to it after recurrent nerve section alone. However *had* they been greatly adducted, even the small amount of abduction gained in these dogs would have reduced the air resistance significantly because the same increase in the width of the rima will result in a much greater opening of the glottis, in proportion, when the cords are close together initially than when they are wide apart. Thus supposing the cords were so adducted that they were 0 65 mm apart, abduction of both cords each by 0 65 mm (the average value obtained) would result in a threefold increase in the width of the rima glottidis. If the cords were already near to the intermediate position such a small degree of abduction would widen the rima by only a small fraction (Fig 2 C)

A number of workers have found in a large porportion of cases a second nerve supply to the cricothyroid, coming from the pharyngeal branch of the vagus and sometimes termed the "middle laryngeal nerve" Lemere (1932) found this nerve in 50% of dogs. Hunt & Kuffler (1954) found it constant enough to investigate the cricothyroid of the cat for evidence of multiple innervation of individual muscle fibres (which they found) and Mårtensson (1966) working on 13 dogs, also studied this subject (but found no multiple innervation) It is reported by Dilworth (1921) that Exner in 1885 observed that in the rabbit, muscle fibres of the cricothyroid did not degenerate unless both nerves supplying it were cut

The evidence in these experiments that the cricothyroids were supplied by only one nerve was their apparent inactivity after it was cut Often beforehand they contracted visibly in expiration they did not do so afterwards and were sometimes pulled medially by the contralateral muscle. If the recurrent laryngeal nerve had previously been cut, section of the solitary external ramus was always followed by complete immobility of the cord.

In these experiments the controversy over *why* the cords so frequently become adducted in recurrent nerve palsies has been ignored It was merely accepted as a clinical fact that such was the case in the human in clinical practice On the basis that the cricothyroid is an adducter and that its paralysis would eliminate one of a number of possible factors responsible for the adducted position, these experiments were designed to provide a quantitative assessment of how much and how often relief from adduction might be expected to take place.

In all of the dogs of this series two findings are constant One is the near intermediate position taken up by the cords in every case of recurrent laryngeal nerve palsy Th other is the only slightly different position found when all the nerves are cut. The latter position is probably determined by factors

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	17		4.78	*s*	5.79	*s*
	18		5.09	*s*	4.83	
	19		6.99	*s*	7 17	*s*
	20[t]		5.42	*s*	5.68	*s*
	21[tt]		4.41	*s*	6.33	
		Before section of both recurrent laryngeal nerves	After section of both recurrent laryngeal nerves		After section of both external laryngeal nerves	
Direct readings	22[t]	7.8 mm	7 4 mm	*s*	8.3 mm	
	23	9.0	9.2	*a*	8.1	*a*
Film readings (later method)	23		9 05	*a*	7 18	*s*

a, average values.
s, single values.
t, all nerves sectioned and readings taken in one operation.
tt, last three nerves sectioned in one operation.

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such as the elasticity of the capsule and ligaments of the cricoarytenoid joint and the slope of its articular surfaces. How much the adduction in a recurrent nerve palsy in the human can be alleviated by inactivating the cricothyroid muscle depends on how much the latter is contributing to the adducted position. This we do not know If it be agreed that like the dog, the intermediate or cadaveric position is the relaxed position of the cords when all the muscles are inactivated, then the adducted position must at least initially be caused by an active process which must result from either recurrent or external nerve impulses (the internal ramus of the superior laryngeal nerve is no longer thought to supply the interarytenoid with motor fibres (Williams, 1951) a finding confirmed in the present series by stimulating the peripheral end of the divided ramus when no changes in the larynx were observed) If the recurrent nerve palsy is *known* to be complete from the start then at the beginning most of the adduction of the cords may be due to cricothyroid contraction and may therefore presumably be modified by inactivation with e.g a local anaesthetic. If on the other hand the recurrent nerve palsy is initially partial or is recovering its function then an imbalance between abductor and adductor tone in the muscles supplied by the recurrent nerve could account for an adducted cord just as much as cricothyroid contraction could The problem here, however is why there should be so often a net increase in adductor tone If the abductor muscles are only responsible for the active abduction further from the intermediate position in inspiration (Tschiassny 1957 Arnold, 1957) then in a partial paralysis the adductors must be overacting rather than the abductors under acting

It is argued that adductor and abductor functions are the resultant of changes in the tone of the opposing muscles just as the position of the limbs is the resultant of agonists and antagonists, and data is then produced showing that the adductor muscles of the vocal cords are heavier than the abductors (Stroud & Zwiefach, 1956) However the analogy breaks down when it is remembered that posture of the skeletal muscles is controlled by reciprocal innervation there is not a tug-of war between flexors and extensors but a balance of tone that is adjusted by a reflex mechanism

There is no reason to think that the balance between adductors and abductors of the *larynx* should not also be under reflex control, although the functions of the two sets of muscles are different and Sherrington (1897) assumed that there was no proprioceptive reflex system there There is evidence in cats that an important part of the afferent feedback is provided by the receptors in the cricothyroid and cricoarytenoid joints, the fibres running in both the recurrent nerve and the internal ramus (Kirchner & Wyke 1965) Mårtensson (1963) applied a stimulu calculated to elicit an afferent discharge from muscle spindles but failed to record any such impulses, although the presence of muscle spindles in considerable numbers in all the muscles of the human larynx is reported by Lucas Keene (1961) None were found in the dog by Mårtensson (1964) who concluded that receptors

activated during muscle contraction are located outside the muscles, mainly in the ligaments and around the joints and that their impulse patterns are "differentiated enough to serve a proprioceptive control mechanism" Assuming a reciprocal innervation between adductors and abductors, co-ordination could be upset by an element of afferent block in a recurrent laryngeal nerve palsy

While it is true that adduction (in the form of a sphincteric action) is the more primitive movement in the larynx (Negus, 1931) and the stronger movement since it is often applied against a high positive intra thoracic pressure it cannot be argued that because a supra maximal tetanic stimulus to the distal end of the recurrent nerve and clinical tetany produce adduction, even when abductors also are in spasm (Stroud & Zwiefach, 1956) the adductor function should therefore predominate in a partial palsy The efferent impulses should be selective in an injured nerve just as they must be in an intact nerve

CONCLUSIONS

Taken either separately or together there can be no doubt that direct measurements and film measurements provide good evidence that cutting the external laryngeal nerve widens the rima glottidis in ipselateral recurrent laryngeal nerve palsy even of four months duration. The case for applying this procedure to human bilateral recurrent nerve palsies with adducted cords therefore had experimental support. In a human patient with complete bilateral recurrent laryngeal nerve palsy it is likely that adduction would be diminished by external ramus section, although the active dilatation in inspiration on exercise would not of course return. In partial recurrent nerve palsies, however some of the adduction may be due to abnormal reflex activity in the remaining functional recurrent filaments.

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ZUSAMMENFASSUNG

Nachdem der linke N. laryngeus recurrens durchschnitten worden war und später ein Schnitt des äusseren Astes des N. laryngeus superior auf der gleichen Seite erfolgte, wurde die Weite der Glottis-Spalte (rima glottis) bei 14 Hunden gemessen Direkte Messungen, die mit Zirkeln gemacht worden waren wurden mit indirekten Messungen von einem Kinofilm verglichen und eine Zunahme in der Weite der rima glottis in 14 Hunden festgestellt (bei Anwendung der einen oder anderen Messungsmethode) Die Anwendung einer Messungsmethode zeigte bei drei Hunden keine Zunahme Bei einem Hund konnte die Erweiterung der rima glottidis nach einer Pause von vier Monaten zwischen den

zwei Operationen festgestellt werden. Eine viel kleinere Versuchsreihe die mit Hunden mit doppelseitiger Lähmung des N. laryngeus recurrens unternommen wurde verlief ergebnislos. Die Möglichkeit der Anwendung einer ähnlichen Technik zum Zweck der Inaktivierung des menschlichen Ringschildknorpelmuskels (cricothyroid muscle) bei Lähmungen des N laryngeus recurrens, steht zur Diskussion.

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THE EFFECT OF TOPICAL ANESTHESIA ON INTERNAL LARYNGEAL BEHAVIOR

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Ultra-high speed motion picture laryngeal photography was employed on four subjects to determine the possible effects on the behavior of the internal larynx f orally administered topical anesthesia. Various parameters of laryngeal behavior were i vestigated, with a d without nesthesia, and compared. They included maximum glottal width, glottal length, width of glottis spuria, duration of openi g, closing, and closed phases of the vibratory vcle and glottal area as a function of time. Results were consistent and failed to demonstrate that pplicati n of topical anesthesia affects the behavio of the larynx.

Although usually not necessary in clinical examination of the larynx, a light topical anesthesia, orally administered by means of an atomizer or cotton swab, is occasionally used Topical anesthesia has also been employed in both direct and indirect photography of the larynx. A common side effect of the anesthetic is an almost instaneous flood of mucus, some of which gathers in the pyriform sinuses of the larynx. Also, certain extrinsic laryn geal muscles may be lightly anesthetized, and possible modifications of internal laryngeal behavior might result, either from damping effects of the mucus, or from a change in the status of the muscle tissue These problems have been recognized by Bjuggren (1960) who employed topical anesthesia to facilitate phase determinable laryngeal photography

Provided topical anesthesia does not produce a change in the behavior of the internal larynx, there are times when its use will facilitate accommoda tion of the laryngeal mirror by the sensitive subject, or patient although its use is rarely indicated, and certainly not advisable except in an appropriate medical environment. In order to justify selected use of topical anesthesia, or equally important, comparison of data, where anesthesia has been used in some instances, and not in others, the possible effect on the behavior of the larynx should be investigated. Therefore, the following experiment was conducted.

METHODS

Subject selection

Four highly trained female subjects, thoroughly familiar with high peed laryngeal photography were selected on the basis of the following criteria

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1 The ability to provide the experimenter with an adequate view of the interior of the larynx
2 Freedom from any apparent voice defects.
3 No prior abnormal reaction to cocain or its derivatives.

The subjects were within an age range that represented young adulthood (19–22) and were probably free from tissue changes due to aging effects.

Equipment

The instrumentation included a rather conventional high speed photographic installation in which the camera, timing devices, and experimenter are all enclosed by a sound treated booth. Such an arrangement helped eliminate camera noise which tends to contaminate recordings made at the time of film exposure, and in addition minimized the effect camera noise may have had on the performance of the subject.

The light source for the photography consisted of a considerably over driven 5000-watt incandescent lamp condensing lens, and a water cell for infra red absorption The timing light on the Fastax camera was driven by a 1000 Hz square wave pulse One hundred foot reels of Tri X reversal film were used

The intensity level of subject phonation was controlled by means of a voice-operated intensity monitor which in essence is a three-position relay held in an intermediate position when the voice intensity is at the criterion level With the relay in this position a green light located within the visual field of the subject was illuminated A similar light wired in parallel with the subject s light panel was located within the camera booth Phonation at a level 2 dB higher or lower than the criterion level caused the relay to activate either a red light indicating excessive intensity or an amber light, indicating insufficient intensity Calibration of the monitor was accomplished by the use of a Bruel & Kjær Sound Level Meter Model 2203 Two high-quality magnetic recorders were utilized One controlled by a voice-operated switch was used to make voice recordings from which measures of vocal pitch were made and also to provide a tape from which a loop could be made. Recordings of the subjects own voice were played from a pre-recorded tape loop on the second recorder through a single ANB III headphone during the course of the experiment.

The equipment for film analysis included a Kodak Analyst frame by frame projector with a lens-to-screen distance that provided a linear optical gain over the actual subject dimensions, of about 3.35 Glottal area measurements were obtained by the use of a Keuffel & Esser compensating polar planimeter

Experimental procedures

The experiment was conducted in three phases or conditions In condition 1 a high speed film was made of the larynx of a subject phonating at her preferred pitch and intensity level The intensity of phonation, during a

practice trial just prior to the film exposure was used to establish an intensity criterion for the remainder of the experiment. A magnetic recording, made during the period when the film was being exposed, was used during the second condition of the experiment. In condition 2, the subjects were asked to duplicate their performance of condition 1. The film exposure rate for a particular subject was determined by the vocal pitch selected during practice trials just prior to filming. Films were exposed at about 4000 frames per second for the lower pitched subjects and at speeds as high as 5850 frames per second for the high pitched subjects. Although one risks inadequate film exposure at higher filming rates, the number of sampling per cycle is increased, and greater resolution of the vibratory characteristics of the vocal folds is obtained.

In condition 2 of the experiment, the subject monitored her pitch by means of the single headphone from a tape loop of her phonation that was made during the filming sequence of condition 1. Intensity of phonation was controlled by the pre-set monitor. When it seemed that the experimental conditions of pitch and intensity were being met by the subject, a second film was made at the same exposure rate as in condition 1. A magnetic recording was also made and was used to determine the pitch of phonation or since the rate of vibration was approximately steady state the fundamental frequency of the voice.

After each filming, the subject was given a rest period, during which the tail end of the film was developed to determine if the shot was satisfactory. In two instances of a total of twelve filmings, a re-take seemed necessary.

Phase three of the experiment was essentially the same as phase two, except that the subject was given a light topical anesthetic (5% cyclaine hydrochloride) administered orally via an atomizer. While the anesthetic was taking effect, the camera was prepared for a final filming. When the subject reported a loss of sensation as determined by light palpation in the oro-pharyngeal region, the third phase was conducted. With the subject again monitoring her pitch level from the tape loop made in condition 1, a third film was exposed at the same rates as during conditions 1 and 2.

In two of the four subjects, conditions 2 and 3 were reversed in order to confound a possible order artifact. Regardless of the order in the experiment, however, the topical anesthesia condition is labeled condition three in further discussion, and in the illustrations.

In all instances but one the entire experiment was conducted in a single session. After the final film had been made and test developed, a fourth film was taken of a grid calibrated in 2-mm units. All conditions of the optical system were left the same as during the experiment, and the grid was moved into the optical field until it appeared sharp and in focus. (Depth of field is essentially zero.) Projection of the photograph of the grid permitted calculation of the optical gain and magnification of the projected laryngeal image. This is a familiar technique, reported in laryngeal studies as early a 194 by Wm. Fletcher.

Data analysis

Film analysis consisted of single frame tracings, on light blue ditto paper of each frame of three consecutive cycles of vocal fold vibration, taken from the last half of the film

A rather persistent problem, related to studies of the larynx, is the establishment of appropriate criterion measures. Although ultra high speed photography has been employed in laryngeal research for over 20 years, quantitative interpretations of the data have, for the most part, been limited to such simple measures as vocal fold length, and glottal area as a function of time Virtually no attempts have been made to define or describe normal or indeed, abnormal internal laryngeal behavior on the basis of quantitative information obtained from high speed films. In an attempt to determine whether topical anesthesia had a measurable effect on the behavior of the internal larynx various measures were made, from the tracings, and also directly from the projected images of the larynx. They included (1) maximum glottal width, (2) maximum glottal length, (3) maximum width of glottis spuria and (4) the locus of maximum glottal width as measured from the anterior commissure expressed in terms of percentage of total glottal length Also, the relative durations of opening, closing, and closed phases of the vibratory cycle expressed in terms of percentage of the total duration of the cycle were examined. And finally the glottal area for each frame in the cycle was measured for three cycles, and the average value computed and graphed

Fundamental frequency was determined by the use of a phonelograph, or oscillographic recorder the galvanometer of which consisted of a 5-inch oscilloscope with a type P 11 CRT The CRT image was projected, by means of a 50-mm lens, onto a revolving, film wrapped drum. The horizontal sweep of the oscilloscope was defeated, so that the beam was deflected only in a vertical direction Tape recordings made during the filming sessions, plus the master loops were subjected to analysis.

RESULTS AND DISCUSSION

Several limitations render a strictly quantitative interpretation of the data very difficult

Camera speed is not necessarily exactly the same from filming to filming. Line voltage variations, slight differences in the pitch of the film temperature of the camera and so forth, may affect filming speed The data in each phase of thi experiment therefore are subject to normal camera speed variations of about 2 per cent

Successive cycles f vocal fold vibration may vary somewhat either because of differences in camera speed or small cycle to cycle changes in vocal fold mechanics (perturbations) In addition, the subjects may not have met the experimental condition exactly with resultant slight variations in vocal fold vibration and intensity of the voice These sources f error and others,

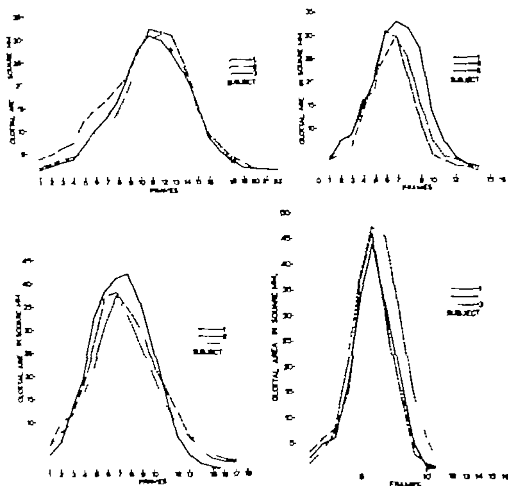


Fig. 1 a–d. Glottal waves (glottal area as function of time) for subject 1 through 4. Glottal area (Y axis) reflects optical magnification by factor f three

may offset one another or they may conjoin to produce differences that may exceed differences attributable to the topical anesthesia.

On the assumption that experimental error does not exceed the experimental effect, it is reasonable to suppose that if a difference attributable to the topical anesthesia exists, then the difference in laryngeal behavior between conditions 1 and 3 or between conditions 2 and 3 of the experiment ought to exceed the differences between conditions 1 and 2. Because the individual performances of the subjects differ so greatly one from the other it seemed unreasonable to group the data. Rather the interpretations are based on the results obtained from each individual subject.

For exemplary purposes, the results obtained from subject 1 are as follows

The glottal waves for subject 1 are shown in Fig. 1 These curves reflect internal laryngeal behavior as well or better than any other measure which

Table 1 *Maximum glottal widths (in mm) for three experimental conditions*

	Condition		
Subject No	1	2	3
1	3 1	3.0	3.4*
2	3 0	3.0	2.8*
3	4 4	4.5	4.3
4	4 0	3.5	4.3

Indicates a possible experimental effect.

Table 2 *Maximum glottal lengths (in mm) for three experimental conditions*

	Condition		
Subject No.	1	1	3
1	11.5	16 0	15.5
2	14.2	16.0	16.1
3	14.5	12.5	13.3
4	15.0	11 6	15.5

I dicates possible experiment l ffect.

Table 3 *Maximum width (in mm) of glottis spuria*

	Condition		
1	9.5	11 0	10.4*
2	11.0	11.0	14 0
3	11 0	11.3	11 0
4	11 1	10 8	12.0*

Indicates possibl perimental effect.

was made The close similarity between curves strongly suggests a lack of experimental effect For quantitative purposes, cumulative glottal area dif ferences, taken at each data point (frame of film) were measured and com parisons between conditions made The same procedure was followed for each subject Results are shown in Table 5

For sul ject 1 the cumulative glottal area difference between conditions 1 and 2 was 15 4 mm when reduced to absolute values, between conditions 2 and 3 was 10 95 mm² and between 1 and 3 was 7 mm² These differences do not reflect any experimental effect Glottal width, which averaged about 3.3 mm varied from condition to condition at the most by 0 4 mm Glottal length, which averaged 15.3 mm varied at the most by 1.5 mm and the width of glottis spuria, which averaged 10.3 mm, varied at the most by 1.5 mm.

Table 4 *The locus of maximum glottal width as measured from the anterior commissure expressed as percentage of total glottal length*

	Condition		
1	58	54	57
2	46	47	37*
3	50	47	45
4	57	59	65

Indicates possible experimental ffect.

Table 5 *Cumulative differences in glottal areas between experimental conditions 1 and 2 between 2 and 3 and between 1 and 3*

Measurements i square millimeters.

	Conditions		
Subject No.	1 and 2	2 and 3	1 and 3
1	15.4	18.9	7 0
2	19.3	10.0	12.3
3	18.0	11 7	20.3
4	8.3	20.0	15.0*

Indicates possible experimental effect.

Length of the opening phase of the vibratory cycle was 50% in conditions 1 and 2, and 54% in condition 3 None of the differences which were observed can be regarded as significant, nor can they be attributable to condition 3, the condition of anesthesia.

The above values and the proportionate magnitude of differences obtained between conditions are very representative of the values obtained from all subjects in this experiment A slight difference in glottal area, which might be attributable to the experimental effect may be seen in the graph for subject 4 This is also reflected in the cumulative differences found in Table 5 Examination of the film, however revealed that an additional frame was gained during the period when the vocal folds were maximally opened. Such slight phase differences are not uncommon in ultra-high speed films of the vocal folds, and become evident, especially when graphs of consecutive cycles are superimposed, one on the other

As seen in Table 3, the glottis spuria shows very little variation between experimental conditions however subjects 1 and 4 give some indication of an experimental effect. The locus of the maximum glottal width, as shown in Table 4 varied considerably between conditions, to the extent that the value of such information is questionable. These large variations also reflect what seems to be an inherent shortcoming of ultra high speed photography

Table 1 *Maximum glottal widths (in mm) for three experimental conditions*

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Subject No.	1	2	3
1	3.1	3 0	3.4
2	3.0	3.0	2.8*
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4	4.0	3.5	4.3

Indicates a possible experimental effect.

Table 2 *Maximum glottal lengths (in mm) for three experimental conditions*

	Condition		
Subject No.	1	1	3
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2	14.2	18.0	16.4
3	14.5	12.5	13.3
4	15 0	11 6	15.5

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Table 3 *Maximum width (in mm) of glottis spuria*

	Condition		
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2	14.0	14 0	14 0
3	11 0	11.3	11.0
4	11 1	10.8	12.0*

Indicates possible experimental ffect.

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For subject 1 the cumulative glottal area difference between conditions 1 and 2 was 15 4 mm when reduced to absolute values, between conditions 2 and 3 was 16 0 mm² and between 1 and 3 was 7 mm² These differences do not reflect any experimental effect Glottal width which averaged about 3.3 mm varied from condition to condition at the most by 0 4 mm Glottal length, which averaged 15.3 mm varied at the most by 1.5 mm and the width of glottis spuria, which averaged 10.3 mm varied at the most by 1. mm

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of the larynx and film analysis, namely the difficulty in delinlating the upper and lower edges of the vocal folds, especially during the closing phase of the vibratory cycle No other parameters of internal laryngeal behavior vary in an outstanding manner for the condition of topical anesthesia, and a conclusion that administration of a light topical anesthesia does not affect the behavior of the internal larynx seems justified.

ZUSAMMENFASSUNG

Vier Versuchspersonen wurden mit einer Hochgeschwindigkeitskamera photographiert, um die mögliche Beeinflussung von mündlich eingeführter lokaler Betäubung auf den inneren Kehlkopf zu studieren. Verschiedene Einzelheiten des Kehlkopfverhaltens wurden mit und ohne Betäubung studiert und verglichen. Maximale Stimmritzbreite Stimmritzlänge Breite des falschen Stimmritzes, Dauer der Öffnungs- Schliessungs- und Geschlossenen-Phasen des Vibrationsvorganges und die Oberflächengrösse des Stimmritzes abhängig von Zeitverlauf werden einbegriffen. Die Ergebnisse waren dieselben bei allen Versuchspersonen und konnten nicht beweisen dass lokale Betäubung das Verhalten des Kehlkopfes beeinflusst.

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ANWENDUNGSMÖGLICHKEITEN DER THERMOANEMOMETRIE IN DER OTO-RHINO-LARYNGOLOGIE

Prinzipien und Methoden

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Die vorliegende Arbeit untersucht eingehend die Möglichkeiten, die das Thermoanemometer bietet, wie auch das Anemometer mit Film in Aufklärung der noch unbekannten wichtigen Formen des Luftlaufes durch die Nasenhöhle. Die zu diskutierende physische Erscheinung, die das Wesentliche der Methode darstellt, wie auch die Komplikationen, die zu einigen Annahmen der Flüssigkeitsmechanik führen, wurde mit einer beigefügten ausführlichen Beschreibung der zwei Vorrichtungen erläutert, mit Betonung der Vorzüge, die das Anemometer mit Film bietet. Ferner werden die Prinzipen der Schemen der zwei Varianten des Thermoanemometers besprochen, nachdem die Vorzüge dieser Methode beim Studium des Luftlaufes durch die Nasenhöhle festgestellt sind die eventuellen Möglichkeiten der Diagnosestellung durch Verarbeitung der ermittelten Angaben mit Hilfe der Wahrscheinlichkeitsrechnung werden aufgezeigt.

Die Fachliteratur untersucht in vielen Werken detailliert das Problem des Zustandes des durch die Nasenhöhle gehenden Luftstromes, ist aber noch zu keiner allgemein anerkannten Schlussfolgerung gekommen, obwohl die experimentellen Studien, die hierzu durchgeführt wurden, zu einem turbulenten Strömungszustand neigen.

Wenn man von der Hauptidee ausgeht, dass der Sinn des Turbulenzvorganges ein Gemisch von Wirbeln, Massen und Bewegungsquantitäten ist, die durch Pulsationen sowohl der Partikel als auch grösserer Partikelgruppen (Mol genannt) verkörpert werden, so muss man bei den Versuchen, die das Strömen der Luft durch die Nasenhöhle betreffen, feine Forschungsmittel anwenden.

Wegen der äusserst komplizierten Struktur der turbulenten Bewegung sind Forschungen mittels einer speziellen Apparatur die eben die Besonderheiten des Problems berücksichtigen muss, notwendig (Versuche mit Luftströmen)

Diese Apparatur muss die Pulsationen der Geschwindigkeiten und eventuell auch die Schwankungen des Druckes aufzeichnen. Da in der Mechanik der Flüssigkeiten — beim Studium der Struktur der turbulenten Bewegung

innerhalb der Versuche mit Luftströmen — die Methode des Thermoanemometers besonders vorteilhaft ist, glauben wir dass die Anwendung der genannten Methode oder der vervollkommneten Methode des Anemometers mit Film, die von Ling & Hubbard (1956) vorgeschlagen wurde uns wertvolle Hinweise für die Klarung dieser so umstrittenen Frage liefern könnte

Das theoretische Prinzip der Methode

Die thermoanemometrische Methode stützt sich auf die bekannte Tatsache dass der elektrische Widerstand eines Leitungsdrahtes mit der Temperatur des Leiters schwankt.

Diese Schwankung wird folgendermassen ausgedrückt

$$R = R_0(1 + \alpha t) \qquad (1)$$

In dieser Formel ist R der Widerstand des Leiters bei der Temperatur t R_0 ist der Widerstand des Leiters bei 0 C α ist der Schwankungskoeffizient des Widerstandes mit der Temperatur

Das Abkühlen eines Leitungsdrahtes, der beispielsweise in einer Nasenhöhle einem Luftstrom ausgesetzt wird hängt im Prinzip von der Leitungsfähigkeit der Flüssigkeit die darüber fliesst ab also ist das Abkühlen direkt bedingt durch das Abfliessen der Flüssigkeitsmasse die durch das Produkt $\rho\mu$ dargestellt wird, in welchem ρ die Dichte des Flüssigkeitsstromes und μ seine Geschwindigkeit bedeuten. Mit anderen Worten die Temperatur die der an einen elektrischen Stromkreis angeschlossene und durch den elektrischen Strom erwärmte Draht erreicht, ist eine Funktion der Geschwindigkeit der Flüssigkeit die ihn, darüber fliessend, abkühlt.

Sowohl das Erwärmen des Glühfadens als auch die Bestimmung seiner Temperatur von welcher der Widerstand direkt abhängig ist werden elektrisch verwirklicht

Bei der unkompressiblen Strömung ist das Verhältnis zwischen der thermischen Überlagerung Q vom Glühfaden auf das umgebende Medium, der Temperatur des Glühfadens T_f und der Fliessgeschwindigkeit der Flüssigkeit, in der Gleichung von King wiedergegeben

$$Q = (T_f - T)(a + b\sqrt{u}) \qquad (2)$$

in der a und b Kalibrierungskonstanten sind und T die Temperatur bei der das Fliessen stattfindet darstellt.

Im Trägheitszustand ist die Geschwindigkeit der thermischen Übertragung vom Glühfaden Q gleich mit der Wärme die durch die elektrische Streuung hervorgerufen wird, nach dem Gesetz von Joule

$$Q = I^2 R_f \qquad (3)$$

Die Stärke des elektrischen Stromes wird mit einem Amperemeter (gewöhnlich einem Potentiometer) zusammen mit einem Standardwiderstand,

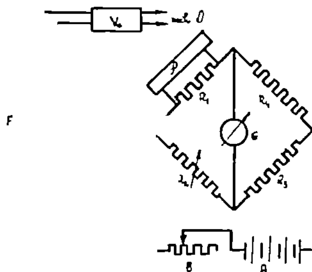


Abb 1 Grundsatzschema des Anemometerglühf dens. *A* Akk mulat rbatteri *B* er änderlicher Widerstand; *F* Draht *G* Gal nomet ; *O* Oszillograph; *P* Potentiometer; *R1 R2 R3 R4* Widerstand *V* Verstärker

der mit dem Glühfaden in Serie geschaltet ist, gemessen, während der Wider stand R_f mit einer Montage (Wheatstonesche Brücke) gemessen wird, wie auf Abb 1 ersichtlich ist.

Die Bestimmung des Widerstandes R_f erlaubt auch die Bestimmung der Temperatur des Glühfadens, unter Verwendung folgender Gleichung

$$R_f = R'[1 + \alpha\,(T_f - T') \qquad (4)$$

wobei R den Widerstand und T' die Referenztemperatur darstellen.

Die Konstanten a und b aus der Gleichung (2) haben für jeden Glühfaden verschiedene Werte, die durch Kalibrierung in einer Strömung, deren Charakteristiken gut bekannt sind, bestimmt werden.

Üblicherweise wird bei der Messung der Durchschnittsgeschwindigkeit bei konstanter Temperatur gearbeitet, durch die Regelung der Stromstärke bei jedem Wert der Geschwindigkeit, genauso viel wie notwendig ist, um den Widerstand R_f konstant zu erhalten

Die Gleichung (2) kann in eine allgemeinere Form umgeschrieben werden, indem man nichtdimensionale Variablen verwendet, und im besonderen durch die Einführung der Zahl n Nusselt

$$N = \frac{Q}{\pi K_f (T - T)}$$

und der Z hl von Reynold

$$\mathrm{Re} = \frac{\varrho u d}{\mu}$$

wobei K und μ die Leitbarkeit und Viskosität der Flüssigkeit bedeuten, während l und d die Länge beziehungsweise den Durchmesser des Glühfadens darstellen

Unter diesen Bedingungen wird das Gesetz von King zu

$$N = A + B\sqrt{Re} \quad (5)$$

Die Universalkonstanten A und B hängen von der Beziehung l/d ab.

Die bedeutendste Anwendung des Glühfadens ist seine Anpassung an ein Anemometer um die Schwankungen der spezifischen Quantitäten einer Strömung zu messen

Der Glühfaden hat den Vorteil dass er in kleinen Dimensionen konstruiert werden kann In diesem Sinne ist es hinreichend zu erwähnen dass der Durchmesser des Glühfadens 10^{-3} mm–10^{-3} ist so dass er schnell und genau auf die Änderungen des Flüssigkeitsstromes und der Temperatur reagieren kann

Wenn die Schwankungen der analysierten Grössen im Verhältnis zu den Mittelwerten der Grössen des betreffenden Stromes klein sind, bewirken sie proportionale Änderungen des Widerstandes oder der Stromstärke

So bewirkt der variable Widerstand des Glühfadens $R f$ beim Handhaben des Schemas, das auf einem Strom mit konstanter Stromstärke beruht, eine variable Voltage

$$e' = I^2R'f = C_1\frac{u'}{u} + C_1\frac{\varrho}{\varrho} + C_3\frac{T}{T_0} \quad (6)$$

Die Proportionalitätsfaktoren C_1 und C_2 werden aus Kalibrierungsdaten erhalten Es ist zu erwarten dass bei der turbulenten unkompressiblen Strömung nur Geschwindigkeitsschwankungen u auftreten, aber bei der kompressiblen Stromung können ausser den Geschwindigkeitsschwankungen auch Schwankungen in der Dichte ϱ und Temperatur t in Erscheinung treten

Folglich beansprucht die Zerlegung des Antwortsignals des Glühfadens *in die 3 Komponenten, die Schwankungen unterworfen sind, ein Ablesen* in drei verschiedenen Betriebsarten Dieser besonders dünne Glühfaden kann nicht mit einer vollkommenen Genauigkeit auf die äusserst schnellen Änderungen der Grössen der turbulenten Stromung antworten, weil die Proportionalitatsfaktoren mit den Frequenzschwankungen n und mit dem Verhaltnis

$$\frac{C(\omega)}{C(o)} = \frac{1}{\sqrt{1 + M^2\omega^2}}$$

abnehmen, wobei $\omega = 2\pi n$ die Schwankungen und M die Zeitkonstante darstellt

Dieser Abschwachung kann man auf zwei Arten Rechnung tragen a) Sie wird bei der Durchführung von Messungen in Betracht gezogen b) Eine passende Einrichtung ist ein Kompensationsamplifikator mit einer Verstärkung, deren Frequenz mit dem Faktor $\sqrt{1 + M^2\omega^2}$ wächst

Die Verspätung der Antwort des Glühfadens ist fast gänzlich auf die thermische Regelung zurückzuführen, weil die für die Strömungsregelung nötige Zeit viel kürzer ist (von der Grösse $d/10^{s}$)

Die Zeitkonstante M kann durch den Vergleich der thermischen Kapazität des Glühfadens mit der verlorenen Wärmequantität, geschätzt werden.

Die genaue Theorie stellt einen Additionsfaktor R_f/Re zur Verfügung, so dass

$$M = \frac{Rf}{\mathrm{Re}} \quad \frac{\varrho_f\, C\, d^2}{K\; Nu}$$

Die Zeitkonstante M für Platin und Tungsten, die gebräuchlichsten Materialen für Glühfäden, ist im Beisein von Luft eine Millisekunde für einen Glühfaden mit der Dicke von 10^{-4} mm. Ling & Hubbard (1956) veröffentlichten unter dem Titel „The hot-film anemometer a new device for fluid mechanics research" das Ergebnis der Forschungen über die Verwendung eines feinen Filmes aus geschmolzenem Platin auf einer Glas- oder Keramik oberfläche, beim Studium der Strömungsmerkmale der Gase und Flüssigkeiten. Seine Handhabung ist derjenigen des Thermoanemometers ähnlich, weil das Funktionieren beider Systeme auf dem Durchfluss eines elektrischen Stromes beruht.

Der Wärmewechsel zwischen dem Film und der Flüssigkeit wird elektrisch als eine Funktion der Strömungsparameter detektiert

Das Anemometer mit Film hat einige höhere Kennzeichen, so dass es in anspruchsvollen Situationen verwendet werden kann Wenn es als ein Übertrager verwendet, sind seine aussergewöhnlichen mechanischen Kennzeichen, die ausgezeichnete dynamische Reaktion und das höhere Sigalisierungsniveau, erwähnenswert Trotzdem bemerkt man, dass bei grösseren Frequenzen die Reaktion des Filmes höher ist, dagegen bei kleinen Frequenzen der Glühfaden eine relativ grössere Reaktion aufweist.

Man muss die Verbesserung des mechanischen Systems der Unterlage des auf ein Glas- oder Keramikfundament gestützten Filmes, zum Unterschied *zu dem auf seinen eigenen Extremitäten gestützten Draht, unterstreichen.*

Auf diese Weise wird der Fehler durch das fremde Signal, das durch die Scherungs- und die örtliche Vibration eingeführt wurde, beseitigt also ist die Richtigkeit der Reaktion hochwertig.

Das Arbeitsschema des Thermoanemometers und die Betriebsweise

Der für die turbulente Strömung kennzeichnende stochastische Charakter der Rührbewegungen macht eine vertiefte statistische Analyse dieser Erscheinung sowohl durch theoretische als auch durch praktische Mittel notwendig

Experimentell könnte die Analyse des Zustandes des durch die Nasenhöhl gehenden Luftstromes, durch Berücksichtigung der jüngsten Entdek

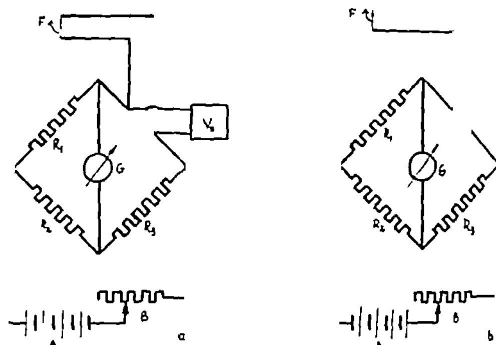


Abb. 2. *a*) Thermoanemometer mit konstantem Widerstand. *b*) Thermoanemometer mit konstanter Voltage. *A* Akkumulatorbatterie; *B* veränderlicher Widerstand; *F* Draht; *G* Galvanometer; *R1 R2 R3* Widerstand; *Vo* Voltmeter.

kungen auf dem Gebiete der Mechanik der Flüssigkeiten, mit Hilfe des Thermoanemometers mit Glühfaden oder Film durchgeführt werden.

Im Prinzip besteht das Thermoanemometer aus einem dünnen, kurzen Draht (mit einem Durchmesser von ungefähr 10^{-3} mm), der aus Leitungsmaterialien mit grossem Widerstandskoeffizienten *a*, gewöhnlich Platin, Tungsten oder Nickel besteht und in einem Wheatstoneschen Brücken Stromkreislauf mit weiteren drei Widerständen montiert wird (Abb. 2).

Wenn elektrischer Strom durch den Draht fliesst, erwärmt sich dieser und wenn er in den Punkt der Nasenhöhle, wo uns die Werte besonderer Kennzeichen des Strömungszustandes interessieren, eingeführt wird, kühlt er mehr oder weniger ab, was entsprechende Änderungen seines elektrischen Widerstandes zur Folge hat. Die Strömungsgeschwindigkeit des Luftstromes oder die analysierte Grösse wird vorher mit bestimmten elektrischen Grössen, die aufgezeichnet werden, korreliert.

Da der Widerstand des Drahtes von seiner Temperatur abhängt, die ihrerseits durch die Strömungsgeschwindigkeit der Luft durch die Nasenhöhle bedingt ist, ergibt sich, dass die Stromstärke selbst von der Geschwindigkeit der Luft in der Nasenhöhle abhängt.

Im Schema auf Abb. 2 sind *R1*, *R2*, *R3* elektrische Widerstände die im Wheatstoneschen Brückenschema mit dem Widerstand *F* montiert sind, das heisst, der Draht des Thermoanemometers ist ein veränderlicher Widerstand. *A* ist eine Akkumulatorbatterie und *G* ein Galvanometer.

Es gibt mehrere Thermoanemometertypen. Der Typ mit konstantem Wi-

derstand (Abb. 2 *a*) und der Typ mit konstanter Voltage oder Intensität (Abb. 2 *b*)

Im System mit konstantem Widerstand ist der Erwärmungsstrom ständig mit Hilfe einer elektronischen Ausstattung adjustiert, um den elektrischen Widerstand *F* konstant zu erhalten.

Die Voltage der Brücke wird so verändert, dass der Zeiger des Galvanometers auf der rechten Seite des Nullpunktes zu stehen kommt. Wenn die Widerstandsveränderungen, also die Temperatur zu vernachlässigen sind, ist ein Ausgleich für die thermische Verspätung nicht nötig.

Das Ablesen auf dem Voltmeter ist mit der Strömungsgeschwindigkeit der Luft durch Kalibrierung korreliert

Das System des Thermoanemometers mit konstanter Voltage ist auf Abb 2 *b* dargestellt.

Die Spannung entlang der Brücke wird konstant erhalten, nach dem der Stromkreis so adjustiert wird dass das Galvanometer Null anzeigt (die Brücke ist im Gleichgewicht) wenn der erwähnte Draht in die Luft im Ruhezustand gestellt wird Wenn die Luft strömt, kühlt der erwärmte Draht ab, der Widerstand ändert sich, und der Zeiger des Galvanometers wird abgelenkt. Die Ablenkungen des Galvanometers sind mit der Luftgeschwindigkeit korreliert.

Die Thermoanemometer mit konstantem Strom benötigen weniger komplizierte Kontroll- und Verstärkungsstromkreise und sind für Messungen der Turbulenzstärke mit niederem Niveau günstiger

Die Verstärkungs- und Messungsstromkreise die mit dem Thermoanemometer in Verbindung gesetzt werden, müssen effektiv auf alle Komponenten der Frequenz der Schwankungen, die von Bedeutung sind, reagieren

Was die Vorteile des Thermoanemometers für das Studium des Strömungszustandes der durch die Nasenhöhle gehenden Luft anbetrifft konnen wir folgende Schlussfolgerungen ziehen

1 Wegen seiner kleinen Dimensionen ist das Anemometer mit Draht oder der vervollkommnete Typ, und zwar das Anemometer mit Film äusserst günstig für Messungen von mittleren Geschwindigkeiten in beschränkten Räumen und in der Nähe einiger Punkte wie z. B. des oberen, mittleren und unteren Meatus.

2 Die Methode des Thermoanemometers ermöglicht die Messung der Richtung des durch die Nasenhöhle gehenden Luftstromes wie auch die Messung der Zusammensetzung der Rührgeschwindigkeit nach einer Senkrechten auf die Richtung der Transportgeschwindigkeit.

3 Man kann Messungen der Schwankungsgeschwindigkeiten durch die Aufzeichnung der Änderungen der elektrischen Spannung mit Hilfe eines elektrischen Spannungsverstärkers durchführen

4 Die für die Turbulenz charakteristischen Grössen und sog Spektralfunktionen φ (*n*) werden experimentell bestimmt mit dem Thermoanemometer das die Schwankungen aufzeichnet, die dann durch ein System elektrischer Filter analysiert werden

5. Sie konnen für die Messung der mittleren Temperatur und der Temperaturschwankungen verwendet werden, was entweder durch sehr kleine Strömungen, bei denen der Erwärmungseffekt vernachlässigbar ist, oder durch zwei verschiedene Erwärmungsströmungen erzielt wird.

Versuche mit dieser Vorrichtung auf Grund einer grossen Menschenzahl konnten Ergebnisse zeitigen die mit Hilfe der Wahrscheinlichkeitsrechnung verarbeitet, die Diagnostizierung verschiedener spezifischer Erkrankungen des Organs ermöglichen könnten

SUMMARY

The paper presented here analyses in detail the possibilities which the hot wire anemometer and hot film anemometer offer us for solving the essential aspects shown by the question of the air flow working condition through nasal fosses. The discussion of the physical phenomenon which represents the essence of the method and at the same time the discussion of the implications encountered in some fluid mechanics, are followed by a detailed description of both devices after that the advantages of the hot-film anemometer are stated. Subsequently the diagrams of connections of both kinds of anemometers and their different readings are discussed then the advantages of this method, applied to the study of the air flow through nasal fosses, are shown and the possibilities of determining the diagnosis with the processing of data by means of the calculus of probabilities are also suggested.

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Acta oto-laryngologica 68, 188–188 (1969)

OPTICAL METHOD FOR DETECTION OF EARDRUM MOVEMENTS

Preliminary Report

T Brask

From the University Department of Otolaryngology Kommunehospitalet Aarhus, Denmark

A preliminary report is gi en of a new optical method for detection of eardrum movements. By this method one can record stapedius and tensor tympani muscle reflexes and measure their lat ncies—with both homo- and contralateral stimulation. These measurements can be per formed equally w ll if there is a perforation in the eardrum.

The detection of middle ear muscle reflexes plays an increasing role in oto-neurological diagnostics. For these measurements different methods exist

(1) Registration of changes in the acoustic impedance of the eardrum.
(2) Registration of changes in air pressure in the closed ear canal caused by eardrum movements.
  (a) Extratympanic manometri.
  (b) Extratympanic phonometri.
(3) Electromyography—by means of electrodes in the middle ear muscles.
(4) Registration of changes in the sound transmission through the middle ear caused by the contractions of the middle ear muscles, when a constant sound is applied to the skull

This change can be established by
  (a) Change of the measuring tone in cochlea (subj method)
  (b) Change of the sound pressure in the closed ear canal (obj method)

Investigations of these methods are in progress in our laboratory
(5) Optical methods
  (a) Direct inspection of movable structures in the middle ear
  (b) Application of a micro-mirror to the eardrum—and recording the angular displacement of the mirror

Determination of the latency of the middle ear muscles has been per formed through

(1) Electromyography
(2) Extratympanic phonometri
  Extratympanic manometri
(3) Recording of changes in the acoustic impedance.

5 Sie können für die Messung der mittleren Temperatur und der Temperaturschwankungen verwendet werden, was entweder durch sehr kleine Stromungen, bei denen der Erwärmungseffekt vernachlassigbar ist, oder durch zwei verschiedene Erwärmungsstromungen erzielt wird.

Versuche mit dieser Vorrichtung auf Grund einer grossen Menschenzahl könnten Ergebnisse zeitigen die mit Hilfe der Wahrscheinlichkeitsrechnung verarbeitet, die Diagnostizierung verschiedener spezifischer Erkrankungen des Organs ermoglichen könnten

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The paper presented here analyses in detail the possibilities which the hot wire anemometer and hot film anemometer offer us for solving the essential aspects shown by the question of the air flow working condition through nasal fosses. The discussion of the physical phenomenon which represents the essence of the method and at the same time the discussion of the implications encountered in some fluid mechanics, are followed by a detailed description of both devices after that the advantages of the hot-film anemometer are stated. Subsequently the diagrams of connections of both kinds of anemometers and their different readings are discussed then the advantages of this method, applied to the study of the air flow through nasal fosses, are shown and the possibilities of determining the diagnosis with the processing of data by means of the calculus of probabilities are also suggested.

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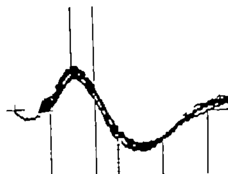


Fig 3

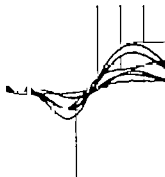


Fig. 4

Fig 3 Recording f stapedius reflexes from normal ear Stim lus t ne-burst f 1000 Hz 100 dB HL. X-a i 20 ms/di Y-a i 0.5 mV/di The nset f signal (reflex) i about 18 ms (lat ncy) after th onset f th sweep (stim lus onset)

Fig 4 Record ng of tensor tympani reflexes from eardrum with big perf ration. Stim lus short rapid ai jet tow rd one f the orbital regi ns. X-axi 20 m div Y-axi 1 mV/di Lat cy is bout 65 ms. The spread f the tracing is due t purposely produced intensity variations f the stimul s.

A model set-up has been devised in which the distance from the measuring probe to a test object can be controlled The photoconductive cell is series connected to a voltmeter which indicates a voltage depending on the amount of light falling on the photoconductive cell The meter reading as a function of the probe-to-object distance is given in Fig 2 This curve is specific for the particular probe geometry but the gross shape is general to the measuring principle A high sensitivity to distance variations from a given position means a great slope of the curve The highest sensitivity appears to be in the range A (distance 0–0 7 mm.) Next to this is range "C" (1.5–4 mm) with a nearly constant slope and sensitivity while range "B" shows a low and rapidly changing sensitivity

When the probe is applied to an ear it will generally be difficult to utilize the range "A" (0–0 7 mm) due to the shape and angular position of the ear drum So the measurements should be carried out in the range "C" (1.5–4 mm) Movements smaller than 10^{-3} mm can be recorded in this range and thus the instrument is very suitable for recording movements of the eardrum caused by contraction of the tympanic muscles.

The measuring probe is fixed in a device which can close the ear canal airtight. In this way it is possible to change the air pressure in the ear canal and to make homolateral acoustic stimulation.

In order to determine the latencies of the middle ear muscles, the stimulus —eliciting the reflex—is used as the triggering pulse for the oscilloscope (sweep onset) The latency i the time interval between the onset f th sweep and the onset of the signal (reflex) Because of noise it can be diff cult exactly t assess the onset of the signal from a single tracing, fo which

reason a series of repeated tracings are recorded on the oscilloscope with a camera attached. The photograph will in this way display a number of superimposed tracings, and this bundle of traces allows an improved assessment of the latency

From the direction of the onset of the signal one can decide whether the eardrum has moved towards or away from the measuring probe in the initial movement though the course of the whole curve is not portraying the object movements truly—due to electrical filtering

The measurements can also be done if the eardrum is perforated Further one can record eardrum movements caused by externally applied sound.

ACKNOWLEDGMENT

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ZUSAMMENFASSUNG

Ein vorläufiger Bericht von einer neuen optischen Methode für die Bestimmung der Bewegungen des Trommelfells wird gegeben. Bei dieser Methode können die Reflexe und die Latenszeiten der Reflexe von dem Stapedius und Tensor Tympani Muskel registriert werden Auch wenn das Trommelfell perforiert ist können die Registrierungen gemacht werden

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Had undvej 21
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A CLINICAL METHOD FOR DETERMINATION OF NASAL AIRWAY RESISTANCE

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Quantitative determinations of nasal airway resistance (*Rn*) must be made at specified flow rates ($\dot{V}$) through the nose. The most reliable method to determine *Rn* is when $\dot{V}$ and the pressure drop across the nose (*Pn*) are recorded simultaneously during spontaneous breathing. A new rhinomanometric method using a flow regulator is described. The values of *Rn* obtained from this method have been compared to those obtained from simultaneous recordings of *Pn* and $\dot{V}$ on the same subjects.

Ten healthy persons and a number of rhinitic patients were examined with both methods and very good agreement was found between the results of the two methods. Furthermore, the new method gave information on abnormalities of vascular reactions in nasal diseases when other methods do not. The transducer and recording system were later exchanged for a liquid or mechanical manometer which makes the new method very convenient for clinical use.

Quantitative determinations of nasal airway resistance, *Rn*, are of considerable clinical interest. *Rn* is defined as the quotient between the pressure drop across the nasal cavities and the flow rate. As *Rn* varies with the flow rate (Lilly 1950, Butler 1960, Ferris *et al.* 1964, Speizer & Frank, 1964, Craig *et al.* 1965, Solomon & Stohrer 1965) only methods where *Rn* is determined at a specified flow rate through the nasal cavities can give adequate information and will be discussed in this paper.

Symbols

$\dot{V}$ = air flow rate through the nose in litres per second (LPS)
Pao = pressure at the airway opening (nares)
Pm = pressure in the mouth.
Prh = pressure in the rhinopharynx.
Pn = pressure drop across the nose (Pao – Prh)
(All pressures are given in cm H_2O.)
Rn = airway resistance between rhinopharynx and nares (cm H_2O/LPS)

This work was supported by grants from the Medical Faculty, University of Lund, and from Alfred Österlund Foundation, Malmö, Sweden.

Previous methods

There are three different possibilities to determine *Rn* from *Pn* and $\dot{V}$

1. *Pn* and $\dot{V}$ are allowed to vary and must be determined simultaneously
2. *Pn* is kept constant and $\dot{V}$ or the volume passing the nose during a given time is determined
3. $\dot{V}$ is kept constant and *Pn* is determined

This principle of classification is the best for a description of available methods.

Ad modum 1 This method is from the physiological point of view superior to other methods as determinations may be done at a normal, spontaneous breathing pattern $\dot{V}$ is as a rule determined by a pneumotachograph, but also rotameters or body plethysmographs have been used *Pm* is assumed to equal *Pth* *Pn* is measured as the differential pressure between the mouth and a sealing mask applied over the nose and connected to the flow meter (Fig 1 A) *Several investigations have been based on this principle* (Lehmann 1939 Lilly 1950 Aschan *et al.*, 1958 Semerak 1958 Butler 1960 Cottle, 1960 Guillerm *et al.*, 1961 Klajman & Sitkowski, 1961 Ferris Jr *et al.*, 1964 Craig Jr *et al.*, 1965 Masing, 1965 Solomon & Stohrer 1965) Spoor (1965) measured nasal conductivity as the quotient $\dot{V}/Pn$

Ad modum 2 This principle has been applied in different ways (Kayser 1895 Zwaardemaker 1909 Curtner 1911 Undritz, 1930 Winslow *et al* 1934 Scheideler 1939) The method is not suitable for many purposes as it demands extreme cooperation, such as relaxation of the soft palate In some modes of application it demands catheterization of the rhinopharynx breathholding and an artificially produced air flow through the nose

Ad modum 3 Some methods based on this principle have been described (Sternstein & Schur 1936 Scheideler 1939 Dishoeck, 1942 Seebohm & Hamilton 1958 McLaurin *et al.*, 1960 Ingelstedt & Ivanderantz, 1961 The constant air flow through the nasal cavity is produced by blowing or sucking air through the nose The flow rate is regulated in different ways. In such applications the principle shares some of the drawbacks connected with methods *ad modum* 2 According to our experience some patients will fail to relax the tongue and the soft palate in the way required Such failure will cause erroneous determinations, as will be discovered when comparisons are made with a method *ad modum* 1

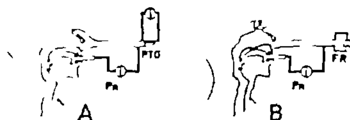


Fig. 1 (A) The arrangement for determination of *Rn* using the pneumotachograph *PTG* method and (*B*) using the flow regulator *FR* method.

It is thus quite evident that methods *ad modum* 1 i.e. simultaneous de terminations of V and *Pn* during spontaneous breathing, are superior to other methods described so far The demand for rapid, simultaneous deter minations of flow and pressure however makes such methods less suitable in clinical routine use outside the research laboratories.

It is our opinion that when designing rhinomanometric methods for clini cal use they *should be tested against a method ad modum 1* used as refer ence method. No such comparisons seem to have been performed in the same subjects in order to test other methods.

The aim of the present investigation was to design and test a new method for accurate determinations of *Rn*, simple and suitable for clinical use The method to be described below which is based on breathing through a flow regulator also allows studies of variations of nasal resistance at different transmural pressures of the mucosal vessels. Such factors have been shown to affect *Rn* in patients with diseases of the nasal mucosa (Runderantz, 1964) No method suitable for routine examinations of this factor has been available so far

METHOD

The present method is based on the determination of *Pn* during spon taneous breathing at a flow rate settled by a flow regulator (FR) The method should thus be classified *ad modum* 3 The FR is attached to a mask covering the nose in such a way that it does not distort the nasal shape, Fig. 1 *B* The FR, modified and described by Jonson (1969) only allows a known constant flow of air to pass independently of the breathing effort of the patient, i.e. independently of the pressure in the mask as long as this pressure exceeds 2 cm H_2O *Pn* is determined by a differential pres sure manometer connected to the nosemask and the mouthpiece.

Corresponding techniques based on flow regulation of spontaneous respi ration have earlier been described for determinations of pulmonary resist ance (Allander *et al.*, 1964) and of the resistance of trachea and larynx (Ingelstedt & Jonson, 1967)

Technical Details

Fig. 1 *A* shows the principal arrangements of the reference method *ad modum* 1 The flow (V) was determined by a pneumotachograph (Fleisch no 2) connected to a pressure transducer (EMT 32, Elema-Schönander Solna, Sweden) *Pn* was determined by a differential pressure transducer (EMT 34 Elema Schönander) V and *Pn* were registered against time on a recorder (Mingograph 81 Elema Schönander) To make it easier to keep a free air contact between mouth and rhinopharynx a mouthpiece was used which kept the incisors 15 mm apart, but allowed the lips to seal around the connecting tube. The arrangements for the FR-method are shown in Fig 1 *B* The FR was attached to the mask thus replacing the flow meter

In the comparative study between the methods, *Pn* was consistently determined by the above-mentioned transducer system. However the FR method does not demand instantaneous determination of *Pn* as the flow rate is kept constant throughout the breath which lasts for 4–6 seconds. Therefore the pressure transducer and recording system can be exchanged for a leaning liquid or a *mechanical differential manometer*[1]. *In a form suitable for* clinical use the FR method thus does not demand any electronic device.

A second mechanical manometer was connected to the nose mask to check that the patient's respiratory effort was great enough to produce the pressure needed for the FR to regulate the flow. This pressure was also recorded on the Mingograph.

SUBJECTS AND PROCEDURE

The comparisons between the two methods mentioned were performed in 10 healthy volunteers, six women and three men aged 23–31 and one man 72 years old. They had no story or clinical signs of disease affecting the nose. Rhinoscopy showed no abnormalities.

Furthermore a number of patients suffering from nasal diseases was also *included in this investigations in order to demonstrate the possibility of* studying different vascular reactions of the nasal mucosa.

Each healthy subject was examined on two occasions with several days interval. The following procedure was followed. Determinations of *Rn* were performed at expirations and inspirations through the FR set at a flow rate of 0.33, 0.50, 0.67 and 0.83 LPS. *Rn* was also determined by the method *ad modum* 1 (PTG method) and calculated at corresponding flow rates. Moderate hyperventilation was needed to reach the higher flow rates. For each subject the order of the two methods was reversed at the second investigation.

When using the FR method the pressure within the nasal cavities is dependent on the respiratory effort. As this pressure acts from the outside on the vessels within the nasal mucosa, mucosal congestion and *Rn* could be expected to vary according to the respiratory effort. In order to examine this factor the volunteers were instructed to make respiratory efforts so as to create a pressure in the nasal cavities varying between 5 and 25 cm H_2O positive or negative during expirations and inspirations respectively. These pressures were determined from the nasal mask and could be read on a manometer by the subject. Each inspiration and expiration had a duration of 4–6 seconds.

The patients were examined in the same way. In order to exclude reasons for variations of *Rn* others than mucosal engorgement the patients were given nosedrops (1% ephedrine solution) 10 minutes before a second examination.

[1] The equipment I f rm itable for ll kal se will be ll ll from Fl m Schö nder Solna, Swed

All the investigations were performed in an air conditioned room with a constant temperature of +19 C (±0.2 C) and with a relative humidity of 60% (±5%)

RESULTS

The healthy subjects

No difference between inspiratory and expiratory resistance was found with any of the methods. Some 76 values according to the pneumotachograph method (Rn_{PTG}) and to the flow regulator method (Rn_{FR}) have been available for statistical analyses. Each value was a mean of 3–4 expirations and inspirations.

The comparison shows that Rn_{PTG} on an average is 0 1 cm H_2O/LPS lower than Rn_{FR}. The difference is significant ($0\ 005 > p > 0\ 001$) [Mean of $(Rn_{FR} - Rn_{PTG}) = 0\ 096$ s.D $= 0.29$ s.E. $= 0\ 033$] Fig. 2 shows Rn_{PTG} and Rn_{FR} at different flow rates in the normal subjects. The mean values and 1 and 2 standard deviations are shown The small difference between $Rn_{\ R}$ and Rn_{PTG} shown above exists at all flow rates but decreases with increasing $\dot{V}$ The resistance increases at increasing flow rate, and Rohrer s formula

$$R = K_1 + K \quad \dot{V}$$

is well suited to describe the present results.

Thus,

$$Rn_{PTG} = 0.55 + 1.51\ \dot{V}$$

and

$$Rn_{FR} = 0\ 76 + 1.31\ \dot{V}$$

The lines drawn in Fig 2 correspond to these equations. The spread of Rn in the group of normal subjects is similar for both methods as shown by the coefficient of variation. The majority of the subjects showed good agreement with the observations of Rn on different days. Some subjects, however showed considerable variations of Rn from day to day variations which were revealed by both methods.

The error of the methods defined as the standard deviation of the differences between duplicate determinations was 0 45 and 0.25 cm H_2O/LPS for the PTG method and the FR method, respectively

The patients

Recordings performed at breaths of normal duration and at moderate intranasal pressure (5–10 cm H_2O) have been analysed statistically Some 62 comparisons between Rn_{PTG} and Rn_{FR} show that Rn_{PTG} is on average 0.2 cm H_2O/LPS higher than Rn_{FR} but the difference is not significant ($0.2 > p > 0\ 1$) [Mean of $(Rn_{FR} - Rn_{PTG}) = -0\ 167$ s.D $= 0\ 93$ s.E. $= 0\ 118$] No difference between inspiratory and expiratory resistance was found.

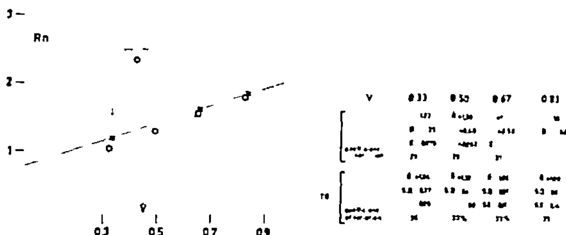


Fig. 2. Th me of R f 10 healthy lunteers t diff rent fl w rates ccordi g t the FIt d the PTG methods. The lines show Rn according t R hrer formul $(Rn = K + K$ $\dot{V})$ 1 nd 2 st ndard devi ti n are ill strated. The t ble how mean f Rn $(\bar{R})$ th st ndard deviati (s.p.) the st ndard erro (s.e.) and the coefficient f vari ti n f Rn at different fl w rates d methods.

Effect of Different Pressures Within the Nasal Cavities

The results were consistent in all ten healthy volunteers. Fig 3 illustrates the findings in one of them The effect of a pressure of -20 cm H_2O during a prolonged inspiration caused very little or no increase of Rn (range 0–0.9 cm H_2O/l/S) During forced expiration the corre ponding decrease of Rn was 0–0.3 cm H_2O/l/S.

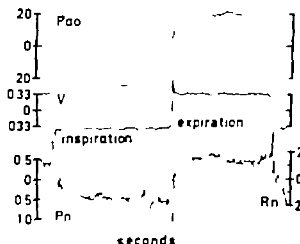


Fig. 3. Prolonged pow rf l i pirati nd pirat n f he lthy bject t pres ure l the ose m k Pao of nd 20 m H_2O The con t t flow t nd the tran na l pres re P lso pressed I ev lst ne R re ltn l t neously recorded N or i thi se little incre se f R nd Pn occ rred t normal duri g the piration

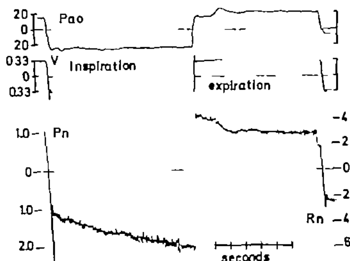


Fig. 4 Observ tion correspo ding t those of Fig 3 in patient with allergic rhi itis. Rn changes considerably d ring the respiratory manoeu res.

Fig 4 shows the findings in a patient suffering from allergic rhinitis. When a constant negative pressure was prevailing within the nose during an inspiration for 8 seconds *Rn* increased from 3 4 to 6 0 cm H O/LPS. During the following expiration with the equivalent positive intranasal pressure, *Rn* rapidly decreased to a stable value of 3 cm H_2O/LPS. 10 minutes after administration of ephedrine nose drops *Rn* was 0.8 cm H O/LPS and did not change at all at varying intranasal pressures. In several other patients with allergic, vasomotor and infectious rhinitis, the same phenome non was observed.

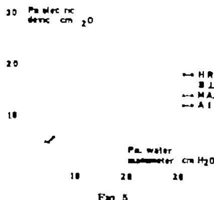


Fig 5.

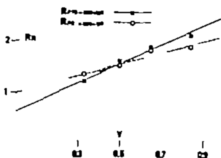


Fig. 6.

Fig. 5. Simult neous determination of *Pn* t constant V with liquid manometer nd with the lectric transducer system. Drawn line is line of identity

Fig. 6. The results of one subject illustrat that even minor ariations of *Rn*, within the range of error of the methods, may result in great variati ns f *K* and *K*

Simultaneous but independent determinations of *Pn* with the liquid manometer and with the transducer system were performed at different flow rates between 0.33 and 0.07 LPS in four subjects. The data obtained are shown in Fig. 5. Extremely good agreement between the two ways of measuring *Pn* was noted.

DISCUSSION

Nasal airway resistance equals the quotient between the pressure drop and the flow rate through the nose. It has long been established that nasal resistance increases with increasing flow rate. Thus, meaningful determinations of *Rn* should be made at a defined flow rate. Only such determinations can be used in comparisons between subjects and in diagnostics of nasal disease.

It is today generally accepted that the most reliable method to determine *Rn* is when V and *Pn* are recorded simultaneously during spontaneous breathing. The need for complicated instruments has restricted its use in clinical routine examinations. Other methods aiming at simplification of the equipment and of the determinations are based on artificial blowing or sucking of air through the nose in order to keep V or *Pn* at a known constant value. The use of such methods is limited as a large proportion of patients cannot cooperate e.g. by breathholding and by relaxation of the tongue and soft palate during the artificial conditions at the determination. By regulating the flow rate during spontaneous respiration those disadvantages may be reduced. The present method includes such regulation which is accomplished by a simple flow regulating valve replacing the flow meter. This arrangement not only keeps the flow rate at a constant pre-set value but also eliminates the demand for rapid determinations of *Pn*. Thus pressure transducers can be replaced by simple mechanical or liquid manometers, which give the same information as shown above. The flow rate can be pre-set at any value between 0 and 1 LPS. Like other authors (e.g. Aschan *et al.* 1958, Craig *et al.*, 1965) we are of the opinion that intranasal instrumentation must be avoided during rhinomanometry because so many errors are introduced by such manipulations. We have used a nose mask of design similar to that of Aschan *et al.* and Craig *et al.*

Sternstein (1941) claimed that if *Rn* of the whole nose is measured, even a complete occlusion of one side may escape detection. We agree with Masing (1965), Craig *et al.* (1965) and others that the whole nose as well as each single cavity should be examined. One nostril may easily be occluded by e.g. adhesive tape without any influence on the other cavity that is being examined. In the present investigation the measurements were performed solely on the whole nose. Our method gave resistance values 0.1 cm H_2O/LPS higher than the reference method in the healthy subjects. This difference is not readily explained. However, it is so small that it may be neglected in clinical work. In patients with nasal diseases there was no such difference.

TABLE 1

Author	Number of subject	Rn cm H_2O/LPS ($\dot{V}$ = 0.5 LPS)	K	K
Butler 1960	5	inspir / expir 2.56 (0.96 – 6.60)	—	—
Ferris *et al.*, 1964	9	inspir 2.78 / expir 2.57	1.09 ± 1.89 / 1.67 ± 1.87	2.18 ± 2.77 / 1.41 ± 1.65
Speizer & Frank, 1964	9	inspir 2.30 (1.34 – 4.45) / expir 2.98 (0.91 – 5.62)	0.62 (0.29 – 1.19) / 0.78 (0.30 – 1.73)	3.42 (1.15 – 6.68) / 4.30 (1.27 – 9.53)
Craig *et al.*, 1965	13	inspir 2.48 ± 1.03 / expir 2.24 ± 0.94	0.9	2.8
Present authors	10			
PTG method		inspir / expir 1.32 ± 0.45	0.55	1.51
FR method		inspir / expir 1.39 ± 0.40	0.78	1.21

In the present investigation no difference of *Rn* at inspiration and expiration was observed with any of the two methods. Some authors claim that *Rn* is greater during inspiration (v. Dishoeck, 1942) others that it is greater during expiration (Speizer & Frank, 1964) Finally some have found no difference—as in this investigation—or a very small one (Craig *et al* 1965) Some published data of *Rn*, *K* and *K* in normal subjects from the last decade are seen in Table 1

Our values of *Rn* obtained by both methods are lower than those found by other authors. This difference may be explained by different principles of selecting the normal subjects. We have excluded all subjects showing a pronounced pressure-dependent nasal congestion. Such subjects generally have a higher nasal resistance than our group of normal subjects and a thorough penetration of the story of such subjects nearly always reveals some kind of nasal trouble.

Breathing through a flow regulator creates artificial, effort-dependent pressure variations in the nose. Pressure variations from +20 to −20 cm H_2O during prolonged expirations and inspirations, respectively caused little or no change of *Rn* in normal subjects. The interpretation is that the mucosal vessels resist such variations in transmural pressure and change their volume only slightly resulting in little or no change of nasal resistance. In patients with rhinitis of varying etiology the resistance often changed considerably in such conditions. This phenomenon was abolished by local administration of vasoconstrictive agents. Together with the fact that the changes of *Rn* occur within seconds this was regarded as evidence that the observed increase of resistance at subatmospheric intranasal pres-

sures was caused by the development of vascular congestion. Whether such congestion is due to a primarily low tone of the vascular smooth muscles or if it is due to a lack of increase of tone at increasing vascular transmural pressure, cannot be evaluated from the present results. The effect of ephedrine proves, however that the vessels under appropriate conditions can develop a tone great enough to resist an increased transmural pressure No other method offers the possibility of detecting the above discussed pattern of vascular reactions.

The choice of flow rate for clinical investigations of nasal resistance has been widely discussed. Solomon and Stohrer claimed that *Rn* should be determined at a flow rate not below 0.5 LPS at which flow rate turbulence may be expected to be established. Several authors (Butler 1960 Guillerm *et al.*, 1961 Craig *et al.*, 1965 Solomon *et al.*, 1965) have also made their determinations of *Rn* at the flow rate of 0.5 LPS which occurs during normal breathing at rest. The spread of *Rn* values in normal subjects, measured as the coefficient of variation, is about the same at different flow rates between 0.33 and 0.83 LPS (Fig. 2) Thus no special flow rate is to be preferred from this point of view Further clinical studies may show at which flow rate pathological phenomena are best revealed.

Determinations of *Rn* at different flow rates allow calculation of K_1 and K of Rohrer's formula. Even minor variations of the results within the error of the method cause however great variations of the calculated K_1 and K_2 (Fig. 6) As a consequence of this K_1 and K_2 seem to be of little clinical use in single subjects. In a group of subjects or in a single especially carefully studied subject where random errors have little influence, Rohrer's formula may however be well suited to describe the variation of *Rn* at different flow rates.

The observed increase of *Rn* at increasing flow rate has been shown by others as mentioned above Such an increase could result from increasing Bernouilli forces on the mucosa at increasing flow which factor was stressed by Gray 1967 It was shown above that in normal subjects intranasal pressure changes had little effect on *Rn*. Therefore it seems highly unlikely that small lateral pressure changes caused by the Bernouilli phenomenon at normal breathing would have such effects in normals. The likely and generally accepted reason for increasing *Rn* at higher flow rates is therefore turbulence of the air flow The importance of such turbulence for air conditioning within the respiratory tract has been stressed by Ingelstedt & Toremalm (1960)

Determinations of nasal airway resistance at breathing through a flow regulator according to the above described principles give accurate information, though the method is simple with respect to instrumentation and performance. Furthermore it may give information on abnormalities of vascular reactions in rhinitic patients which other methods fail to do. For these reasons we believe that it could be of value in clinical routine use.

ZUSAMMENFASSUNG

Quantitative Bestimmungen des Nasenwiderstandes (*Rn*) müssen bei bestimmten Strömungsgeschwindigkeiten der Luft ($\dot{V}$) durch die Nase gemacht werden. Das Einführen von intranasalen Instrumenten soll vermieden werden. Eine neue rhinomanometrische Methode, die einen Luftflussregulator ausnützt, wird beschrieben Die mit dieser Methode gefundenen *Rn*-Werte wurden mit denjenigen verglichen, die man bei simultaner Registrierung vom Druckfall des Luftstromes und von $\dot{V}$ bei derselben Personen erhielt Zehn gesunde Versuchspersonen wurden untersucht. Die Untersuchungen ergaben eine sehr gute Übereinstimmung zwi schen den Resultaten der beiden Methoden. Die neue Methode ergab ausserd m Informationen über abnorme Gefässreaktionen in der Nasenschleimhaut bei einigen Patienten mit Rhinitiden, was bei anderen Methoden nicht der Fall ist. Es hat sich erwiesen dass das von uns benutzte elektronische Messungs- und Regi strierungssystem gegen ein Flüssigkeitsmanometer ausgetauscht werden kann, wa die neue Methode für de täglichen Gebrauch in der Klinik sehr geeignet macht.

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REPRODUCIBILITY OF DURATION OF POSTACCELERATORY NYSTAGMUS

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Two groups, each consisting of 22 subjects, were rotated in both the clockwise and counterclockwise direction in three successive identical accelerations. The strengths of stimulus used were 1 2, 4 and 8 °/sec² The duration of nystagmus (denoted as the *after-discharge*) was measured after each acceleration. It was found that the mean duration was longest in the first test, and then decreased successively on repeated stimulation. The decrease was most pronounced at 1 and 2°/sec² The interindividual variation was consistently slightly less at the higher strengths of stimulus, with a concurrent marked decrease in the intraindividual variation. The mean difference was small in a comparison between the results of clockwise and counterclockwise acceleration on repeated stimulation. The calculated borderline values show that the difference between clockwise and counterclockwise acceleration was smallest at the higher strengths of stimulus. This also applied to the calculated borderline values in the second and third tests. Large individual deviations were, however observed. It is discussed whether the pendulum function of the cupula–endolymph system may be correlated to the nystagmus reflex. It is concluded that the nystagmus reflex may possibly reflect the movements of the cupula–endolymph system at accelerations of 1–4 °/sec² but that the central nervous influence results in shorter after-discharges than could be expected at accelerations of 4–8 °/sec²

In an earlier study of post-acceleratory nystagmus, we found that the duration of nystagmus was longer with increasing strength of stimulus (Fluur & Mendel, 1968 *a*) The object of the present investigation was to ascertain the reproducibility of the post-acceleratory nystagmus on repeated, similar tests in the same individual.

CASE MATERIAL

The experimental subjects consisted chiefly of medical students, and the remainder of nurses and nurses assistants. All were aged 20–40 years, and in good general condition. Their hearing was normal, and they had no history of any disease that might have affected the results. It was also checked that they had not taken any medicine on the days preceding the tests.

APPARATUS FOR STIMULATION

The subject sat in an electrically driven rotation chair with his head secured in a holder and inclined 30 forward All the experimental arrangements and recording conditions were the same as in our previous investigations (Fluur & Mendel 1966)

MODE OF STIMULATION

The experiments were divided into two groups. These comprised the following strengths and duration of stimulation

Group 1	1 /sec^2 for 40 sec	*Group 2*	4 /sec^2 for 30 sec
	2 /sec^2 for 40 sec		8 /sec^2 for 20 sec
	4 /sec^2 for 30 sec		

The examination was carried out as follows. The subject took his seat in the rotation chair and the electrodes and lightproof diving mask were put on The procedure was explained to him, and a test acceleration was made at about 2 /sec^2 to check that everything was functioning, and also to give the test subject an idea of the nature of the experiment.

One experiment in group 1 started from a constant speed of about 5 /sec^2 after which we accelerated clockwise at 1 /sec^2 for 40 sec, followed by a constant speed for a few minutes The chair was then decelerated at 1 /sec^2 for 40 sec, after which a constant speed followed for a few minutes. This acceleration-deceleration test was repeated twice more This was succeeded, in an analogous way by stimulation at 2 /sec^2 for 40 sec, and 4 /sec^2 for 30 sec.

The experiments were made in a similar way in group 2, at 4 /sec^2 for 30 sec, and 8 /sec^2 for 20 sec.

DISTRIBUTION OF THE MATERIAL AND CALCULATIONS

The members of the two groups were distributed as far as possible at random Both group 1 and group 2 contained 22 subjects. Obviously the same subject could be included in both groups, but in these cases at least a few days elapsed between the experiments.

We denoted the duration of the postacceleratory nystagmus as the *after discharge* and this was measured after each stimulation with an accuracy of ± 1 sec We calculated the standard deviation and the coefficient of variation of each subject s results in the three tests with the same strength and direction of stimulus (Fluur & Mendel, 1966) In addition, the results in each of the two groups were analysed in a similar way as shown on the right hand side of the tables.

RESULTS

A survey of Tables 1–5 shows that the standard deviation at the different strengths of stimulus decreased with rising strength of acceleration. The mean values of the different individual standard deviations are given at the bottom of each table These S' values decreased from 7.23 and 5 61 (in clockwise and counterclockwise stimulation, respectively at 1 /sec²) to corresponding values of 3 01 and 3 46 at 8 /sec² At 1 /sec² a difference was present between the results in clockwise and counterclockwise stimulation, in that the S' value 7.23 in the former is higher than the S' value 5.61 in the latter At the other strengths of stimulus, the results were approximately the same in acceleration in both directions.

The various individual variations are apparent from the C values (C = coefficient of variation) the C' value being the arithmetic mean of the individual C values at each strength of stimulus. A distinct decrease in the C' values occurred when the stimulus was increased. At 1 /sec² the C' value was 0 40 in clockwise acceleration, and 0.31 in counterclockwise and at 2 /sec² it was 0.27 in acceleration in both directions. At 4 /sec² the values were 0 16 and 0 18 in clockwise and counterclockwise acceleration, respectively (group 1) the corresponding values at 8 /sec² being 0 13 and 0 12.

The variation between the different subjects is apparent from the S values on the right-hand side of the tables. It is seen that these values differed in the two groups. In group 1 they changed inappreciably with an increase in strength of stimulus. In group 2, on the contrary a distinct decrease occurred with a rise in strength of stimulus from 4 to 8 /sec²

A successive decrease in the C'' values took place with an increase in the strength of acceleration. At 1 /sec² the mean values for the three tests were 0.55 in clockwise acceleration, and 0 45 in counterclockwise. On an increase to 2 /sec² the corresponding values were 0 46 and 0.38 and at 4 /sec² they were 0.36 and 0.33, respectively (group 1) Acceleration at 8 /sec² gave mean C'' values of 0.19 It can also be seen in the tables that the S'' and C'' values were by no means the same at 4 /sec² in groups 1 and 2.

If we turn to the individual results, it is seen that at 1 /sec² clockwise acceleration, the smallest individual coefficient of variation was 0.07 and the largest 1 05 The corresponding values for counterclockwise acceleration were 0.09 and 1 00 At 2 /sec² the coefficients of variation were of the order of magnitude of 0 05–0 74 in clockwise acceleration and 0.02–0 60 in counterclockwise Clockwise acceleration at 4 /sec² resulted in individual coefficients of variation ranging from 0.05–0.35 the corresponding values at 4 /sec² counterclockwise acceleration being 0 02–0.68 (group 1) In group 2, acceleration at 4 /sec² produced approximately the same result. Clockwise acceleration resulted in coefficients of variation ranging from 0.03–0.33, the corresponding value in counterclockwise acceleration being 0.04–0 44

TABLE 1

No. of acc.	Case 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Clockwise acceleration 1°/sec²														
1	21	7	9	75	35	12	11	27	15	17	30	23	22	19
2	22	9	0	33	27	14	14	25	11	15	26	21	11	33
3	19	18	4	27	42	24	10	34	13	15	20	18	12	12
X	20.7	11.3	4.3	45.0	34 7	16.7	11 7	28.7	13.0	15.7	25.3	20.7	15.0	21.3
S	1 48	5.87	4.52	26.15	7 48	6.42	2.13	4 74	2.00	1.23	5.00	4.23	6.08	10.68
C	0 07	0.52	1.05	0.58	0.22	0.38	0.18	0.17	0.15	0.08	0.20	0.20	0.41	0.50
										X' = 20.5		S = 7.23		C' = 0.40
Counterclockwise acceleration 1°/sec²														
1	9	23	8	18	26	29	19	31	6	17	24	36	10	24
2	11	19	13	5	27	37	17	32	14	10	30	29	21	19
3	6	18	12	16	17	19	15	27	11	15	20	23	22	23
X	8.7	20 0	11 0	13.0	23.3	28.3	17 0	30.0	10.3	14 0	24.7	29.3	17 7	22.0
S	2.51	2.63	2.65	.00	5.48	9.00	2.00	2.65	4.06	3.61	5.00	6 48	6.67	2.63
C	0.29	0.13	0.24	0.54	0.24	0.32	0.12	0.09	0.39	0.26	0.20	0.22	0.38	0.12
										X' = 19.8		S' = 5.61		C' = 0.31
Difference between after-discharge in clockwise and counterclockwise acceleration														
1	+12	−16	+ 1	+57	+ 9	−17	−8	−4	+9	±0	+6	−13	+12	− 5
2	+11	−10	−13	+28	± 0	−23	−3	−7	−3	+5	−4	− 8	−10	+14
3	+13	± 0	− 8	+11	+25	+ 5	−5	+7	+2	±0	±0	− 5	−10	−11

Finally it can be seen that the individual coefficients of variation were still lower at 8°/sec². Thus, they ranged from 0 02–0.33 in clockwise acceleration, and from 0 02–0.32 in counterclockwise.

In the tables showing the individual results, it is seen, in addition, that the values in the repeated tests in the respective subjects varied in a way that is hard to survey. To obtain an idea of whether repeated tests produced a displacement towards longer, unchanged or shorter after-discharges, we can study the X'' values in the first, second and third tests at each strength of stimulus. It is found that at 1°/sec² the mean after-discharge (X'') was 23.5 sec after the first clockwise acceleration, and 18.8 and 18 7 sec after the two following ones. Thus, the mean after-discharge was much longer in the first test. Counterclockwise acceleration gave approximately the same mean values in the first and second tests, whereas the third test was associated with an average decrease of 5 sec.

At 2°/sec² the mean values in the three clockwise accelerations were

15	16	17	18	19	20	21	22	X''	S''	C''	$K\ S$	$\frac{X-K}{S}$	$\frac{X+K}{S}$
14	10	41	32	16	17	18	47	23.5	15.56	0.66	41.97	−18 43	+65.51
12	9	17	25	25	33	9	23	18.6	9 12	0 49	24.60	− 5.78	+42.42
21	11	14	15	29	28	7	18	18.7	9 08	0.49	24.62	− 5.84	+43.20
								20 3	*11 16*	*0 44*	*30 37*	*−10 1*	*+50 7*
15 7	10.0	24.0	24.0	23.3	26 0	11.3	32.7						
4.74	1.00	14.80	8.64	6.63	8.19	5.87	21.21						
0.30	1.00	0.62	0.36	0.28	0.32	0.52	0.65						
5	29	26	32	21	18	27	28	21.2	8.85	0.42	24 14	− 2.96	+45.32
10	34	32	51	18	14	17	16	21.6	11 07	0.51	29.84	− 8.22	+51.50
0	9	22	16	8	17	25	22	16.5	6.71	0 41	18.10	− 1 60	+34.60
								19 8	*8 81*	*0 45*	*24.03*	*− 4 2*	*+42.8*
5.0	24 0	26.7	33.0	15.7	16.3	23.0	22.0						
5.00	13.23	5 00	17.51	6.82	2.06	5.29	6.00						
1 00	0.55	0.19	0.53	0.43	0.13	0.23	0.27						
+ 9	−19	+15	± 0	− 5	− 1	− 9	+19	+2.36	16.28		43.91	−41.55	+46.27
+ 2	−25	−16	−28	+ 7	+19	− 8	+ 7	−2.82	13.89		37 46	−40.28	+34.64
21	+ 2	− 8	− 1	+21	+11	−18	− 4	+2.18	11.20		30.21	−28.03	+32.39

27.3, 23.7 and 24.2 sec, the corresponding values in counterclockwise acceleration being 25.8, 24 4 and 20.5 sec. Some decrease was also observed at 4 /sec² clockwise acceleration, in which the values were 20 4 29.3 and 27 9 whereas those in counterclockwise acceleration were about the same in all three tests (28.6, 27 9 and 28.0 sec) This also applied to clockwise accelera tion in group 2 (28 6, 29 1 and 27 6 sec) whereas the results in counterclock wise acceleration showed a successive decrease in the values (29.8, 27 4 and 24 7 sec) In the three tests at 8/ sec, the after-discharges were consistently of about the some duration.

To permit a survey of the results, we have plotted in Fig. 1 the mean values in all accelerations in both directions at the different strengths of stimulus, in the first, second and third tests. If we start from the curve for the first tests at each strength of stimulus, it is seen that the following test (curve 2) consist ntly resulted in a shorter after-discharge, and that a further shortening had occurred in the third test (curve 3) The thick un

TABLE 2

No. of acc.	Case 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Clockwise acceleration 2°/sec²														
1	25	21	6	41	38	23	17	54	31	24	30	17	17	32
2	24	16	2	16	53	25	25	47	33	33	29	32	19	29
3	18	26	10	27	38	23	17	58	28	31	23	25	20	22
X	22.3	21.0	6.0	28.0	43.0	23.7	19.7	53.0	30.7	29.3	27.3	24.7	18.7	27.7
S	3.74	5.00	4.00	12.53	8.63	1.16	4.58	5.57	2.46	4.69	3.76	7.48	1.50	5.10
C	0.17	0.24	0.67	0.45	0.20	0.05	0.23	0.11	0.08	0.16	0.14	0.30	0.08	0.18
										$X = 25.1$		$S' = 5.54$		$C' = 0.2$
Counterclockwise acceleration 2°/sec²														
1	23	30	16	23	23	34	26	47	27	18	27	27	28	28
2	24	13	10	19	24	26	38	47	42	24	23	26	24	27
3	21	18	22	15	21	20	19	37	27	16	21	39	18	29
X	22.7	20.3	16.0	19.0	22.7	26.7	27.7	43.7	32.0	19.3	23.7	30.7	23.3	28.0
S	1.48	8.72	6.00	4.00	0.58	7.00	9.59	5.79	8.63	4.16	3.00	7.21	5.00	0.87
C	0.07	0.43	0.38	0.21	0.02	0.26	0.35	0.13	0.27	0.22	0.13	0.23	0.21	0.03
										$X = 23.5$		$S' = 5.84$		$C' = 0.27$
Difference between after-discharge in clockwise and counterclockwise acceleration														
1	+2	−9	−10	+18	+15	−11	−9	+7	+4	+6	+3	−10	−11	+4
2	±0	+3	−8	−3	+29	−1	−13	±0	−9	+9	+6	+6	−5	+2
3	−3	+8	−12	+12	+14	+3	−2	+21	+1	+15	+2	−14	+2	−7

broken curve, which gives the arithmetic mean of the three aforementioned curves, shows that the increase in the after-discharge was only 1.5 sec when the strength of stimulus was increased from 4 to 8°/sec².

We calculated the difference between the after-discharge in each clockwise-counterclockwise test. When the after-discharge in clockwise acceleration was longer, the difference, shown at the bottom of each table, is preceded by a plus sign. When the reverse applied, the difference is preceded by a minus sign. The calculated mean difference varied within the range −2.82 to +3.73 sec. It is seen in the tables that these mean differences in the first, second and third test vary in a completely undependable way and a study of the individual differences gives a still more varying picture. The borderline values, which were calculated for the clockwise-counterclockwise difference in the first test, were computed so that 95% of normal values would fall within the limits with 95% certainty. Largely the same borderline values were found in all three tests. Although certain displace-

15	16	17	18	19	20	21	22	X''	S''	C''	$K\ S$	$X-K\ S$	$X+K\ S$
30	15	24	23	35	40	25	32	27.3	10.84	0.39	28.48	− 1.21	+55.75
25	10	14	6	8	35	20	20	23.7	12.56	0.53	33.87	−10.19	+57.55
26	5	19	19	14	37	13	34	24.2	11.21	0.46	30.23	− 6.00	+54.46
								25.1	*11 44*	*0 48*	*30 15*	*5 8*	*+44 8*
27.0	10.0	19.0	16.0	19.0	37.3	19.3	28.7						
2.65	5.00	5.00	8.89	14.18	2.43	6.02	7.55						
0.10	0.50	0.26	0.56	0.74	0.07	0.31	0.26						
12	17	38	13	21	29	35	25	25.8	8.24	0.32	22.22	+3.55	+47.99
7	18	26	11	29	24	20	34	24.4	9.87	0.40	26.62	−2.26	+50.98
20	13	28	4	8	11	12	29	20.5	8.69	0.42	23.41	−2.94	+43.94
								23 6	*8.83*	*0 38*	*24.08*	*−0 5*	*+47 7*
13.0	16.0	30.7	9.3	19.3	21.3	22.3	29.3						
6.56	2.65	6.40	4.75	10.59	9.27	11 66	4 47						
0.50	0.17	0.21	0.51	0.60	0.44	0.52	0 15						
+18	−2	−14	+10	+14	+11	−10	+ 7	+1.50	10.52		28.37	−26.87	+29.87
+18	−8	−12	− 5	−21	+11	± 0	−14	−0.68	11.26		30.37	−31.05	+29 69
− 6	−8	− 9	+15	+ 6	+26	+ 1	+ 5	+2.73	10.53		26.40	−24 67	+32.13

ments can be noted in some cases, these are fairly inappreciable seen against the background of the scattered values in the tables.

DISCUSSION

In this investigation, a prolonged after-discharge was noted with rising strength of stimulus. *The arithmetic means increased by approximately* 50% from 20 1 sec at 1 /sec² to 30 1 sec at 8 /sec² This increase was the subject of a previous paper (Fluur & Mendel, 1968 *a*)

Parallel with an increase in the strength of stimulus from 1 to 8 /sec² we found a successive decrease in S' values, i.e. a decreased individual variation in the duration of the after-discharge There are two possible explanations of the regular course observed with strengths of acceleration around and above 4 /sec² One is that the cupulae are then so strongly resilient that

TABLE 3

No. of acc.	Case 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Clockwise acceleration 4°/sec²														
1	30	17	21	30	26	23	23	63	35	28	25	25	24	40
2	24	16	22	23	24	27	19	67	42	25	28	29	29	41
3	23	29	27	22	22	29	19	73	22	27	25	28	17	41
$\bar{X}$	25.7	20.7	23.3	25.0	23.7	26.3	20.3	61.0	33.0	26.7	26.0	27.3	23.3	41.7
S	3.81	7.23	3.24	4.36	1.53	3.08	2.31	10.58	10.15	1.58	1.73	2.06	6.04	2.08
C	0.15	0.35	0.14	0.17	0.06	0.12	0.12	0.17	0.31	0.06	0.07	0.08	0.26	0.05
										$\bar{X}' = 28.8$		$S' = 4.54$		$C' = 0.16$
Counterclockwise acceleration 4°/sec²														
1	28	21	32	28	25	32	26	57	42	22	24	30	29	31
2	26	19	28	38	14	30	34	42	35	26	27	26	29	34
3	27	50	40	35	23	29	28	47	25	4	22	32	27	38
$\bar{X}$	27.0	31.0	33.3	33.7	20.7	30.3	29.3	48.7	34.0	17.3	24.3	29.3	28.3	34.3
S	1.01	16.64	6.11	5.15	5.87	1.53	4.16	7.65	8.52	11.72	2.65	3.06	1.16	3.54
C	0.04	0.54	0.18	0.15	0.28	0.05	0.14	0.16	0.25	0.68	0.11	0.11	0.04	0.10
										$\bar{X}' = 28.5$		$S' = 4.96$		$C' = 0.18$
Difference between after-discharge in clockwise and counterclockwise acceleration														
1	+2	−7	−11	+2	±0	−9	−3	−4	−7	+6	+1	−5	−5	+9
2	−2	−3	−6	−15	+10	−3	−15	+15	+7	−1	+1	+3	±0	+10
3	−4	−21	−13	−13	−1	±0	−9	+26	−3	+23	+3	−4	−10	+3

they return to the resting position with great regularity. This implies a constant, mechanically elicited decrease in the vestibular nerve impulse balance caused by the greatly deviated cupulae. Another possibility is that the central nervous system strongly counteracts every vestibular imbalance exceeding that obtained at 4°/sec² and above and that—when the cupula swings back—this central effect would immediately come into action, and inhibit the nystagmus reflex.

At an acceleration of less than 4°/sec² the cupular deviation and the resulting vestibulo-ocular imbalance are not so great, and would only result in relatively weak nystagmus. This nystagmus can be envisaged to be easily influenced by the concurrently varying central nervous systems which affect the vestibulo-ocular reflex arc. This provides a possible explanation of the relatively greatly varying after-discharges in repeated, weak accelerations.

The high S' value in clockwise acceleration at 1°/sec² is presumably to be ascribed to certain subjects being somewhat tense in the first test, with

15	16	17	18	19	20	21	22	X''	S''	C''	$K S$	$\frac{X-K}{S}$	$X+K \times S$
39	25	20	42	17	43	24	38	29.4	9.51	0.32	25.65	+3.76	+55.06
32	13	21	33	31	38	29	38	29.3	10.03	0.34	27.06	+2.22	+56.32
23	12	29	26	28	35	23	33	27.9	11.81	0.42	31.85	−3.99	+59.71
								28.9	*10.44*	*0.36*	*28.18*	*+0.7*	*+47.1*
31.3	16.7	23.3	33.7	25.3	38.7	25.3	36.3						
8.03	1.53	4.85	8.03	7.28	4.06	3.24	2.89						
0.26	0.09	0.21	0.24	0.29	0.11	0.13	0.08						
22	23	32	11	18	36	22	36	28.6	9.22	0.32	24.87	+3.77	+53.51
18	23	28	8	34	24	34	37	27.9	8.18	0.29	22.06	+5.85	+49.97
21	19	28	9	33	34	28	36	28.9	10.68	0.37	28.63	+0.03	+57.89
								28.5	*9.34*	*0.33*	*24.24*	*+3.3*	*+63.7*
20.3	21.7	29.3	9.3	28.3	31.3	28.0	36.3						
2.12	2.31	2.34	1.53	8.97	6.44	6.00	0.59						
0.10	0.11	0.08	0.16	0.32	0.21	0.21	0.02						
+17	+2	−12	+31	−1	+7	+2	+2	+0.77	9.62		25.95	−25.18	+26.72
+14	−10	−7	+25	−3	+14	−5	+1	+1.36	10.13		27.32	−25.96	+28.68
2	−7	+1	+17	−5	+1	−5	−3	+1.00	11.18		30.15	−29.15	+31.15

resulting longer more varying after-discharges. This can also be seen from the λ values, which are much higher (23.5 sec) in the first clockwise acceleration than in the subsequent tests. One could expect similar conditions to apply to the first test in group 2. Here, however it seems as if the course of nystagmus is unaffected by such tension factors, so that about the same X'' values are recorded in all three tests. Analogously the C'' values at 4 /sec² are approximately the same in groups 1 and 2.

Inspection of the X'' values at the different strengths of acceleration showed a tendency to a shorter after-discharge in the three successive tests. This applied consistently with the exception of 4 /sec² counterclockwise acceleration in group 1 The tendency to a shorter after-discharge was most pronounced at the weaker strengths of stimulus. This is possibly to be ascribed to the subjects not being so much aware of the stimulation, so that they relaxed more than was the case with stronger stimuli. It is less probable that the observed decrease was due to habituation, since previous investiga

TABLE 3

No. of acc.	Case 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Clockwise acceleration 4°/sec²														
1	30	17	21	30	25	23	23	53	35	28	25	25	24	40
2	24	16	22	23	24	27	19	57	42	25	28	29	29	44
3	23	29	27	22	22	29	19	73	22	27	25	28	17	41
$\bar{X}$	25.7	20.7	23.3	25.0	23.7	26.3	20.3	61.0	33.0	26.7	26.0	27.3	23.3	41.7
S	3.81	7.23	3.24	4.36	1.53	3.06	2.34	10.58	10.15	1.53	1.73	2.08	6.04	2.08
C	0.15	0.35	0.14	0.17	0.06	0.12	0.12	0.17	0.31	0.06	0.07	0.08	0.26	0.05
										$\bar{X}' = 28.8$		$S' = 4.54$		$C' = 0.16$
Counterclockwise acceleration 4°/sec²														
1	28	24	32	28	25	32	26	57	42	22	24	30	29	31
2	26	19	28	38	14	30	34	42	35	26	27	26	29	34
3	27	50	40	35	23	29	28	47	25	4	22	32	27	38
$\bar{X}$	27.0	31.0	33.3	33.7	20.7	30.3	29.3	48.7	34.0	17.3	24.3	29.3	28.3	34.3
S	1.01	16.64	6.11	5.15	5.87	1.53	4.16	7.65	8.52	11.72	2.55	3.06	1.16	3.54
C	0.04	0.54	0.18	0.15	0.28	0.05	0.14	0.16	0.25	0.68	0.11	0.11	0.04	0.10
										$\bar{X}' = 28.5$		$S' = 4.96$		$C' = 0.18$
Difference between after-discharge in clockwise and counterclockwise acceleration														
1	+2	−7	−11	+2	±0	−9	−3	−4	−7	+6	+1	−5	−5	+9
2	−2	−3	−6	−15	+10	−3	−15	+15	+7	−1	+1	+3	±0	+10
3	−4	−21	−13	−13	−1	±0	−9	+26	−3	+23	+3	−4	−10	+3

they return to the resting position with great regularity. This implies a constant, mechanically elicited decrease in the vestibular nerve impulse balance caused by the greatly deviated cupulae. Another possibility is that the central nervous system strongly counteracts every vestibular imbalance exceeding that obtained at 4 /sec^2 and above and that—when the cupula swings back—this central effect would immediately come into action, and inhibit the nystagmus reflex.

At an acceleration of less than 4 /sec^2 the cupular deviation and the resulting vestibulo-ocular imbalance are not so great, and would only result in relatively weak nystagmus. This nystagmus can be envisaged to be easily influenced by the concurrently varying central nervous systems which affect the vestibulo-ocular reflex arc. This provides a possible explanation of the relatively greatly varying after-discharges in repeated, weak accelerations.

The high S' value in clockwise acceleration at 1 /sec^2 is presumably to be ascribed to certain subjects being somewhat tense in the first test, with

15	16	17	18	19	20	21	22	X	S''	C''	K S	(X − K)/S	(X − K)/S
19	23	47	24	20	26	45	28	28.6	7.28	0.25	19.63	+8.96	+48.22
37	18	40	26	21	43	42	28	29.1	7.94	0.27	21.41	−7.68	+50.50
36	28	38	26	20	38	44	22	27.8	7.56	0.27	20.39	+7.25	+48.03
								28.4	*7.59*	*0.26*	*20.47*	*+7.9*	*+48.9*
30.7	23.0	41.7	25.3	20.3	35.7	43.7	26.0						
10.12	5.00	4.75	1.16	0.59	8.75	1.51	3.46						
0.33	0.22	0.11	0.05	0.03	0.25	0.01	0.13						
40	25	41	26	24	25	36	26	29.8	8.45	0.22	17.40	−12.42	+47.22
27	25	36	33	25	23	30	19	27.4	8.23	0.23	16.80	+10.56	+44.16
18	15	38	24	21	17	35	21	24.7	7.12	0.29	19.20	− 5.53	+43.93
								27.3	*6.60*	*0.25*	*17.80*	*− 9.5*	*+45.1*
28.3	21.7	38.3	27.7	23.3	24.7	33.7	22.0						
11.07	5.79	2.52	4.75	2.09	4.18	2.09	3.61						
0.39	0.27	0.07	0.17	0.09	0.16	0.06	0.16						
−21	− 2	+8	−2	−4	+ 1	+9	+2	−1.23	7.45		20.09	−21.32	+18.86
10	− 7	+4	−7	−4	+20	+3	+9	+1.73	8.35		23.06	−21.33	+24.79
18	13	±0	+2	−1	+21	9	+1	+2.91	8.05		21.71	−18.80	+24.02

If the system had reacted according to the pendulum law this after-discharge would have been produced by an acceleration of approximately 5.3 °/sec². If the time constant of the course of the curve between 4 and 1 °/sec² is calculated, we obtain the value of 5.8 sec, which is lower than that reported by van Egmond *et al.* (1949, 1952). They obtained values from 10 to 25 sec in calculation of the time constant of the cupula–endolymph system. However in animal experiments (Ledoux, 1958) the time constants calculated for the cupula–endolymph system were of the same order of magnitude as those obtained by us for nystagmus in man.

It is thus possible that the movement pattern of the cupula is reflected to some extent in the after-discharge following weak stimuli, but not in the acceleration range 4–8 °/sec². Here the reflex system is probably influenced by inhibiting factors, which produce a much shorter after-discharge than that corresponding to the return time of the cupula pendulum.

In group 1 the S'' values were consistently the same at the different

TABLE 5

No. f acc.	Case 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Clockwise acceleration 8°/sec² (Group 2.)														
1	28	35	27	22	21	35	29	23	31	32	31	21	27	40
2	22	33	21	24	33	28	31	27	31	37	27	30	32	36
3	20	33	33	24	22	28	36	29	32	35	24	27	27	28
X	23.3	33.7	27 0	23.3	26.3	30.3	32.0	26.3	31.3	34 7	27.3	26.0	28.7	34.7
S	4 18	1 16	6 00	1 16	5.87	4.06	3.61	3.08	0.59	2.55	3.54	4.58	2.92	6 12
C	0.18	0.03	0.22	0.05	0.22	0.13	0.11	0 12	0.02	0.07	0.13	0.18	0.10	0.18
										$X = 30.4$		$S' = 3.61$		$C' = 0.13$
Counterclockwise acceleration 8°/sec²														
1	19	34	26	20	31	28	27	24	29	41	31	27	33	29
2	21	32	28	29	25	29	26	23	37	34	26	28	31	24
3	23	28	29	26	33	30	28	26	31	35	28	28	32	25
X	21 0	31.3	27 7	25.0	30 7	29 0	27 0	27 7	32.3	36.7	28.3	27 7	32.0	26 0
S	2.00	3.06	1.53	4.58	4.05	5.87	1 00	5.70	4 16	3.61	2.65	0.59	1.01	2.65
C	0 10	0.10	0 06	0.18	0.16	0.20	0.04	0.21	0.13	0.10	0 09	0 02	0.03	0.10
										$X = 29.2$		$S' = 3.46$		$C' = 0.12$
Difference between after-discharge in clockwise and counterclockwise acceleration														
1	+9	+1	+1	+2	−10	+7	+2	−11	+2	−9	±0	−6	−6	+11
2	+1	+1	−7	−5	+ 8	−1	+5	+ 4	−6	+3	+1	+2	+1	+12
3	3	+5	+4	−2	−11	−2	+8	+ 3	+1	±0	−4	−1	−5	+ 3



Fig 1 Mean duration f fter-discharge in three successive t sts. ▲ 1st test ● 2nd t st; 3rd test ○——○ arithmetic mean f value i all 3 tests.

strengths of acceleration. This shows that the members of the group consist ently exhibited about the same differences in relation to each other at these strengths of acceleration The fact that group 2 had another S value is natural, since the subjects were not the same The highest S'' value at

15	16	17	18	19	20	21	22	X''	S''	C''	K S	$\frac{X-K}{S}$	$\frac{X+K}{S}$
33	34	39	31	33	41	39	28	31.0	5.80	0.19	15.64	+15.40	+46.68
33	17	40	35	22	38	38	34	30.4	6.25	0.21	16.86	+13.55	+47.27
37	31	29	30	25	33	37	25	29.3	5.27	0.18	14.21	+15.11	+43.53
								30.4	*5.77*	*0.19*	*15.56*	*+14.8*	*+46.0*
34.3	27.3	39.3	32.0	26.7	37.3	38.0	29.0						
2.35	9.08	0.60	2.65	5.70	4.06	1.01	4.58						
0.07	0.33	0.02	0.08	0.21	0.11	0.03	0.16						
43	27	33	28	26	32	41	22	30.2	6.31	0.21	17.02	+13.16	+47.20
26	14	35	31	26	29	35	29	28.1	5.18	0.18	13.97	+14.12	+42.06
25	21	41	33	28	31	38	25	29.3	4.86	0.17	13.11	+16.16	+42.38
								29.2	*5.45*	*0.19*	*14.70*	*+14.4*	*+43.9*
31.3	20.7	36.3	30.7	26.7	30.7	38.0	25.3						
10.12	6.52	4.18	2.52	1.16	1.53	3.00	3.54						
0.32	0.31	0.12	0.08	0.01	0.05	0.08	0.14						
−10	+7	+6	+3	+7	+9	−2	+6	+0.86	6.85		18.47	−17.61	+19.33
+7	+3	+5	+4	−4	+9	+3	+5	+2.32	4.82		13.00	−10.68	+15.32
+12	+10	−12	−3	−3	+2	−1	±0	+0.06	5.83		15.72	−15.67	+15.77

1°/sec² clockwise acceleration (15.56 sec) presumably depends on the same factors as those mentioned previously in connexion with the corresponding X'' value. A decrease in the S'' values was noted in group 2 when the strength of acceleration was increased to 8°/sec². We have interpreted this as a sign that the relatively strong stimulus in question (8°/sec²) produces a pronounced vestibular effect, so that there is still less expression of the individual differences.

ZUSAMMENFASSUNG

22 Versuchspersonen wurden in beiden Richtungen rotatorisch akzeleriert, wobei drei aufeinanderfolgende identische Beschleunigungen 1, 2, 4 und 8° angewendet wurden. Die Dauer des Nystagmus wurde nach jeder Beschleunigung gemessen. Es zeigte sich, dass die Dauer des Nystagmus durchschnittlich am längsten bei den ersten Testen war, um dann, nach und nach bei wiederholten Stimulationen abzunehmen. Am deutlichsten war die Verminderung bei Stimulation von 1 und 2°/sec². Die intraindividuelle Variation war durchgehend etwas kleiner bei stärke-

ren Stimuli. Gleichzeitig findet man, dass die intraindividuelle Variation bedeutend abnimmt, wenn man die Beschleunigungsstärke erhöht. Der Unterschied war im Durchschnitt klein, als wir die Resultate von Rechts- und Linksbeschleunigung bei wiederholten Stimulationen verglichen, und die berechneten Grenzwerte zeigen, dass die Rechts- und Linksdifferenz am kleinsten bei den stärkeren Beschleunigungen waren welches auch für die berechneten Grenzwerte in den *zweiten* und dritten Testen gilt. Es wurden jedoch grosse, individuelle Abweichungen notiert. Abschliessend wird diskutiert, wie sich die Pendelfunktion des „Cupula-Endolymph-Systems" zum Nystagmusreflex verhalten könnte. Es ist möglich, dass der Nystagmusreflex die Bewegungen des „Cupula-Endolymph-Systems" bei Beschleunigung von 1–4 /sec² widerspiegelt, dass aber eine zentral-nervöse Einwirkung eine kürzere Nachentladung ergibt, als man bei Beschleunigungen von 4–8 /sec² erwartet hätte.

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ELECTRONYSTAGMOGRAPHIC STUDY OF VESTIBULAR APPARATUS IN PARKINSON'S DISEASE BEFORE AND AFTER COAGULATION OF THALAMUS

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Some authors have made an electronystagmographic study of Parkinson's disease this study was made before and after coagulation of thalamus. Therefore we can give an argue in order to confirm (*a*) the existence of vestibulo-thalamical fibres, (*b*) the contralateral predominance of vesti bular fibres or centres in the thalamus zone (*c*) the existence of the "nystagmogeni centre" in this one (This centre was discovered by Lachmann & Bergman and Monnier & Montandon.)

We observed carriers of Parkinson's disease and noticed a lateropulsion following a coagulation in thalamus region. This lateropulsion, which happens rather frequently is contralateral compared with the place of coagulation. Generally it lasts from four days to ten days, rarely two days. At first, it appears after a coagulation occurring in the susthalamic zone.

We thought that this anomaly could be related to vestibular routes and centres situated either near or in the coagulation zone (de Nó, 1933 v Gehuchten 1937 Brodal *et al* 1962 Arslan & Sala, 1956 Bergman *et al.*, 1959 Lachmann & Bergman, 1958 Monnier & Montandon, 1961 Tye Dumont, 1964) at first we examined patients presenting the lateropulsion phenomenon then patients before and after coagulation. We present our results. (Based on the characteristics given by Aschan (1956) and Torok (1955))

MATERIAL AND METHOD

Patients

16 patients after coagulation 20 patients before coagulation 20 patients after coagulation.

The electronystagmographic examination after coagulation was done after the 4th or 7th day postoperatively All these patients presented, from a clinical point of view Parkinson's disease which was unilateral or nearly unilateral Most of the patients were between 50 and 65 years old however some were much younger

Equipment

We used an alternating-current recorder with 2 pens or a direct-current recorder with 4 pens, with a time constant 0.3, and unfolding 15 millimeters/second.

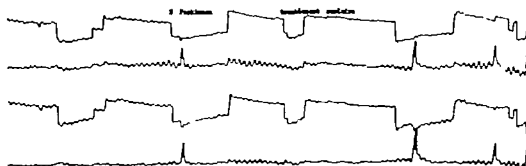


Fig 1 Study of look. Ocular deflections predominating al g the vertical axis.

Examination proceeding

(for each patient and each examination)

(*a*) Examination of eyes ocular deflections, search for ocular tremor search for a spontaneous nystagmus (*b*) caloric test characterized by an injection of 20 centilitres of water at 24 C in 2 seconds, with a qualitative and a quantitative study of the response characters, rhythm aberrant forms, asymmetry between left and right response. Among some patients we made a separate recording for each eye, as we usually make (Pialoux *et al.*, 1966 and 1968) including the data stressed by Jatho (1960)

RESULTS

We studied the results before and after coagulation then the differences between the examination done before operation and that done after operation for the patients who had both.

Results before coagulation

The examination of eyes showed (*a*) a very small ocular tremor which is nearly constant and often irregular and which appears along the horizontal

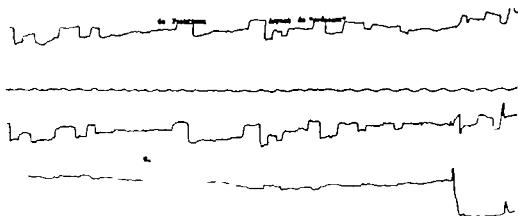


Fig 2. Study f look. Figures in crenel long the horizo tal xis.

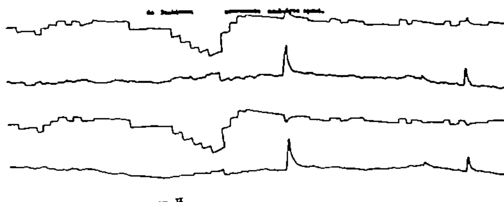


Fig 3 Study of look. Disorder in the voluntary ocular deflections of the eyes along the horizontal axis in "staircase"

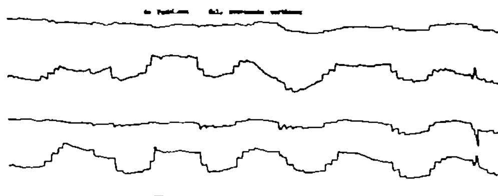


Fig 4 Study of look. Disorder in the voluntary ocular deflections of the eyes along the vertical axis.

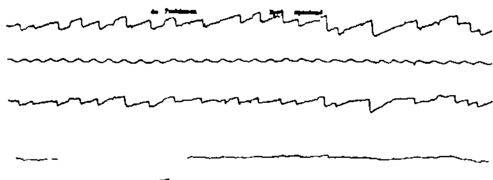
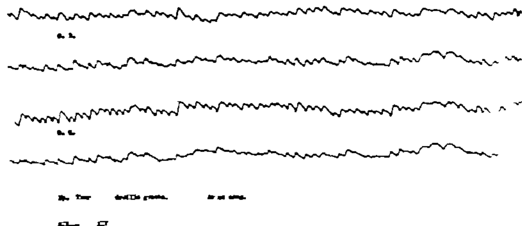


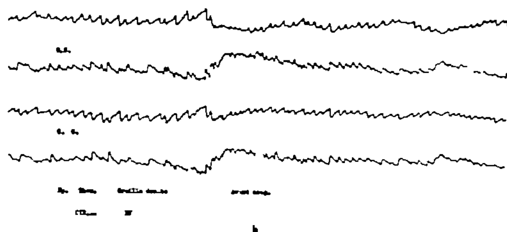
Fig 5 Study of look. Horizontal spontaneous nystagmus with small tremor predominating in the right eye.

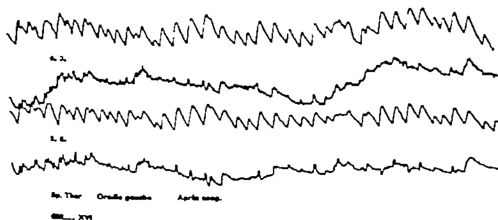
a

b

Fig. 6. Results of caloric tests done 4th day after right thalamic coagulation. There is real asymmetry between the two ears. In the left side (*a*) the amplitude decreased, the frequency increased, and the rhythm is not the same as in the right one (*b*)







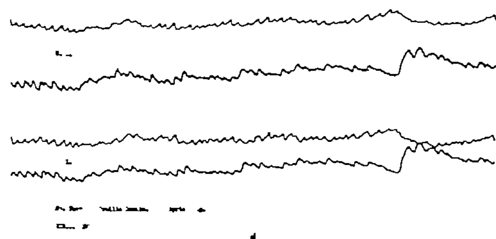


Fig 7 Caloric tests. Before and fter right thalam coagulation. Left ea ( ) nd right ea (*b*) before coagulation. Left ea ( ) and right ear (*d*) fter coagulation (4th day) The more important alterations re l the left side

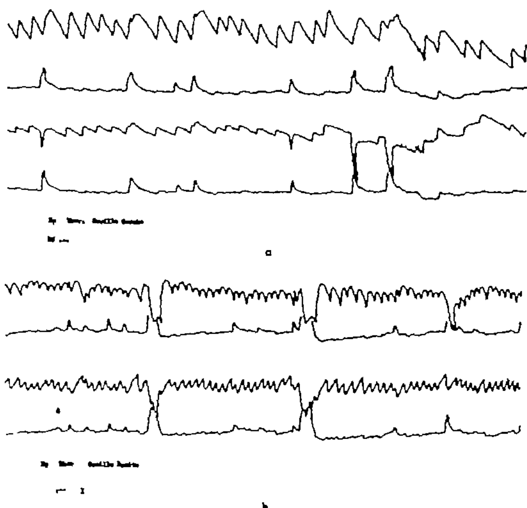


Fig 4 Caloric tests. After left thalamic coagulation. The nystagmus is perturbed in the right side (*b*) and has a pendular aspect. There is an ocular dissociation at the left ear level (*a*) The right eye nystagm shows a more important amplitude than that of the left.

and the vertical axis of the eyes however this phenomenon is more important in the vertical axis (*b*) a constant disorder in the voluntary ocular deflections characterized by a succession of jerks the sum of the amplitudes of these jerks was the same as the amplitude of a single deflection sometimes other jerks follow in an opposite movement the eyes coming back to the anterior position (*c*) some aberrant figures having the aspect of a "crenel" (*d*) *a spontaneous nystagmus, not frequent* (it appears in 4 cases of 20) and nearly always horizontal

The results showed (a) a certain decrease of the middle amplitude which can take the aspect of a "small writing" in one case out of two (*b*) a middle frequency which is nearly the same as *the frequency* usually noticed for a normal patient in most of the cases rarely *fast* enough, excep-

tionally slow (c) a rather normal time but nearly always longer at contra lateral ear level in the site of the lesion (d) a rhythm disorder almost constant characterized by frequency variations and above all by amplitude variations and by the appearance of stops, of tremors and sometimes of aberrant figures in "crenel" (e) the presence of aberrant figures, the jerk having a rounded aspect in one case out of three (f) the frequent presence of an ocular dissociation during the separate recording of each eye characterized by a bigger reaction at one of the eye levels, generally at the homolateral eye, compared with the examined ear and sometimes characterized by the complete absence of reaction at one of the eye levels.

Results after coagulation

The study of the look compared with the first examination shows (a) a small diminution of the tremor (b) the disorder of the ocular deflections that remain (c) the increase of aberrant figures in "crenel" (d) a more frequent appearance of a spontaneous nystagmus which is generally horizontal and in an opposite movement in the place of the coagulation and which is sometimes bilateral

The results, compared each time with the first ones, show (a) same amplitude with, however a small increase of this amplitude (b) same frequency (c) time, rather equivalent, always at the contralateral ear level in the site of coagulation (d) a rhythm disorder which is almost constant and larger than in the first experience above, at the contralateral ear level at the site of coagulation (e) the more frequent presence of rounded figures having even a pendular aspect (f) a more frequent ocular dissociation appearing one time out of two, the nystagmus appearing only at the eye situated on the same side as the site of coagulation (g) an inversion of the nystagmus which in some cases can appear either at both eye levels, or at a single eye level which is homologous to the site of coagulation In the latter case it is characterized by a decrease of the amplitude appearing between the 120th and the 140th second after the beginning of the operation.

DISCUSSION AND CONCLUSION

In Parkinson's disease, the nystagmographic characteristi s seem to be the following (a) disorder of ocular d flections (b) possibility of appearance of aberrant figures in crenel" ( ) possibility of a spontaneous nystagmus, which generally is horizontal (d) normal time for the reaction with, how r a frequent asymmetry th reaction being a little longer in the opposite side of the lesion (e) middle frequency decreased amplitude (f) disorder of the rhythm, with unded figures (g) possibility of an ocular dissociation. These charact istics increase with the coagulation of the susthalamic zone this increase i always greater at the contralateral ear lev l compared with the sit of coagulation. At last, an inversion of the

nystagmus can appear we have to add that the normal characteristics were more important when there was the *lateropulsion* mentioned at the beginning of this study

Therefore we can (1) give an argue in order to reiterate the presence of vestibulothalamical fibres that had been already confirmed by de Nó and Brodal and others. However *we cannot define their situation exactly*

(2) We can give an argue in order to confirm the contralateral predominance of vestibular fibres or centres at this level

(3) We can give an argue in order to confirm the presence of the reticular nystagmogenic centre of Monnier Montandon and of Lachmann & Bergman on account of the rhythm disorder and of the appearance of aberrant figures.

RÉSUMÉ

Les auteurs ont effectué une étude nystagmographique chez des malades présentant une maladie de Parkinson Cette étude pratiquée avant et après traitement par coagulation thalamique apporte un argument clinique *a*) pour confirmer l existence des fibres d association vestibulo-thalamiques *b*) pour confirmer la prédominance contralatérale des fibres au niveau des centres vestibulaires de la région thalamique *c*) pour confirmer l existence du centre nystagmogène de la substance réticulée mis en évidence à ce niveau par J Lachmann & F Bergman et par M. Monnier & P Montandon

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STUDIES ON THE FISSULA ANTE FENESTRAM

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A total of 453 human temporal bones from foetuses, newborns, children, and adults were analysed with respect to the structure and histological appearances of the fissula ante fenestram region in the otic capsule. Our findings differ from those of previous workers on the fissula ante fenestram (Bast, Anson Wilson, and associates) We do not interpret the secondary new-formations of cartilage and bone in the fissula ante fenestram as a precursor of the formation of an otosclerotic focus in this region. The difference between these types of tissue is discussed and elucidated by histological findings.

The area of the bony labyrinthine capsule situated anteriorly to the oval window between the tympanic cavity and the vestibule, has previously been subjected to thorough histological studies, as it has been interpreted as the site of predilection for otosclerosis. It has long been known that at this site there is a minute canal called the fissula ante fenestram (f.a.f) extending from the tympanic cavity through the labyrinthine capsule to the vestibule

Huschke (1884) is said to have been the first to describe the f.a.f in a human temporal bone, but it was Siebenmann who coined its name in 1890 Perozzi, one of Siebenmann's pupils, described its histological structure in 1916 He considered this area to be necessary to the development of the scala vestibuli Mayer's studies from 1917 indicated that f.a.f was a persisting synchondrosis between the primordial cochlear and vestibular parts of the cartilaginous labyrinthine capsule. Guggenheim (1932) after a phylogenetic study considered the f.a.f to be a rudiment of the ductus fenestralis ovalis in the frog

The most important research on the fissular region was done in the 1930's by Anson, Bast, Wilson, and their associates. Their extremely detailed and admirable studies, with numerous histological sections and wax models of the f.a.f are quite unique

To this very day their studies and results concerning the structure shape, contents, and pathology of the fissula have remained, so to speak, undisputed. This also applies to their theory on the role of the f.a.f in the development of the otosclerotic focus. Apart from a publication by Del Bo (1950) supporting this theory there have not been further investigations into the f.a.f region.

Accordingly it might seem unnecessary to return to this subject. However in our studies on the relation of the otosclerotic focus to f.a.f (Bretlau, 1969) we felt that it was indicated to analyse our collection of temporal bones with a particular view to the structure and histological appearances of the f.a.f This analysis has disclosed several items which do not agree with previous studies.

Anatomy

The osseous capsule which encloses the membranous labyrinth is traversed by four fissures of perilymphatic nature viz. the cochlear duct, the vestibular duct the fissula post fenestram, and the fissula ante fenestram. Most interest has been focused on the last mentioned fissure in connection with otosclerosis.

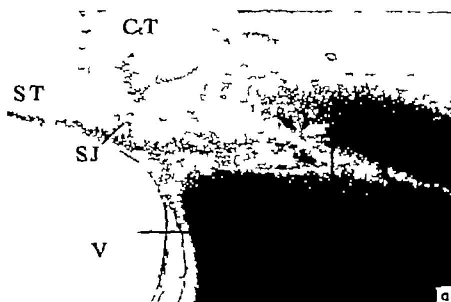
The f.a.f is a constant structure in the human labyrinthine capsule, present also in elderly persons. On the other hand, Bast (1933) did not find it in the ordinary laboratory animals. It extends in the form of a canal through the lateral wall of the labyrinthine capsule Its tympanic opening is in the medial wall of the tympanic cavity above and anterior to the oval window Hence it runs almost in the shape of a C, as a groove or cleft downwards–medially (posteriorly) forming an angle of about 90 and enters the vestibule Its vestibule opening is situated in the angle at the junction of the scala vestibuli and the vestibule

In the foetus and in the newborn infant the f.a.f is usually present in its entirety while in post foetal life its shape and extent are extremely irregular owing to the different histological structures involved. This irregularity is due to the increasing obliteration of the fissula by new formation of cartilage or bony tissue

Embryology

The f.a.f is well developed in a 14-week foetus (100 mm in crown–rump length) but it may be discerned also at an earlier stage (34–50 mm) (Bast, 1933)

The vestibular end of the f.a.f is formed by invasion of mesenchymal connective tissue from the vestibular wall into the precartilaginous labyrinthine capsule in the angle at the junction of the scala vestibuli and the vestibule. Its further development takes place by dedifferentiation of the precartilage with f rmation of areolar connective tissue (Fig. 1) It is by this dedifferentiation of precartilage into areolar connective tissue that the perilymphatic paces in the labyrinthine capsule are formed (Streeter 1918) In the course of this change in th foetal cartilage enclosing the periotic evagination, the fissula increases in length and width. At its tympanic end the mesenchymal tissue later forms a vascular bud which meets the evagination from the vestibule as it invades the foetal cartilage This results in a perilymphatic space from the inner to the middle ear


CT
ST
SJ
V
a


FAF
V
b


CA
FAF
c

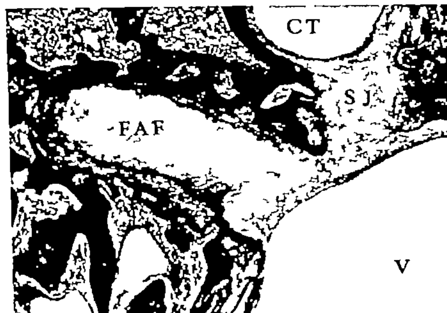


Fig 2 Photomicrograph f normal fl sul ant f estram l newborn with carti-l ge rim surrounded by seous tissue. 40

The f.a.f attains its maximum in the fifth foetal month, corresponding to the 21st week or a C–R length of approx. 180 mm (Bast, 1933)

Histological Structure

The fissular region (Fig. 2) consists of fibrous connective tissue in the f.a.f and the surrounding cartilage and bone of the labyrinthine capsule As a rule the fibrous tissue contains moderately vascularized areolar connec tive tissue reminiscent of and connected with the loose periotic tissue of the vestibule

In a typical f.a.f the connective tissue is surrounded centrally by thin perichondrium whence new formed cartilage reduces the extent of the fissula in its further development. This new formed cartilage is gradually converted into bony tissue of an intra and nchondral type. The areolar connective tissue gradually changes into looser connective tissue in the vestibular end of the f.a.f and into more fibrous connective tissue at its

Abbreviatio in Figs. 1–8. (*CT*) Ca m tympani; (*C*) cochlea (*SJ*) stapedial joint (*PT*) periotic tissue; (*CA*) cartilage; (*EB*) end chondral bone (*V*) estibulum; (*S*) sacculus; (*ST*) st pes; (*FAF*) fissula nt fenestram; (*Ch*) chondroma; (*IB*) intrachondral bone

Fig 1 Photomicrographs f co rse nd relation of the fissula nte fenestram l the cartilaginou otic capsule from foetus f 104 mm C–R length or 14 weeks. ( ) show the fissula extending from the estibule. Detail show the loose areolated nd necrotic tissue. The fi sul enlarges by destruction f the surrounding cartilage ( ) ( ) 25, (*b*) 63, ( ) 250

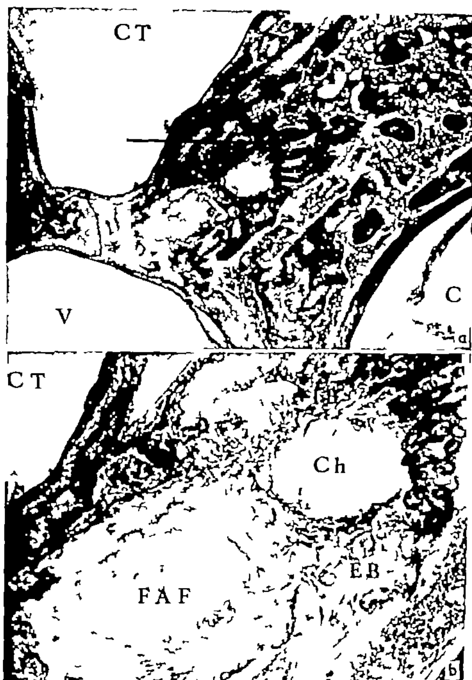


Fig 3 Ph tomicrograph from newborn f th vestibula rifice f th fiss l nt fenestram and cartilage od l (chondrom) situated nea it cochlea end. (*b*) show the difference between th normal cartil ginous shell nd the hondroma l the rea indicated l (*a*) ( ) 25, (*b*) 63.

tympanic end This normal change in structural pattern continues throughout life reducing the f.a f which, however is always discernible, even in elderly persons.

The ossification of the labyrinthine capsule starts in the 16th foetal week



Fig 4 Photomicrograph of chondromatous mass occupying the fissula ant fenestram in newborn, showing the sharp line between the cartilage and the surrounding osseous tissue ×63

and has normally been completed by the 22nd–23rd foetal week, correspond ing to a C–R length of 210 mm (Bast, 1930) This process of ossification is slow in the fissular region, which, however is not the only site of the laby rinthine capsule where some cartilage remains after the ossification. In temporal bones from foetuses and children there may be residual cartilage (Bast & Anson, 1949) at the fissula post fenestram, in the infra-cochlear region, at the base of the styloid process, in the petro-squamous suture and in th labyrinthine capsule just beneath, in the canalicular region and around the round window Thi cartilage may give rise to secondary activity in the form of complete or partial chondrification of the fissular connective tissue resulting in a well-defined cartilaginous mass (chondroma, Fig 4) or in the formation of a neoplastic mass (sclerotic bone) growing beyond the limits of the f.a.f into the surrounding labyrinthine capsule (Fig 5)

This secondary or proliferating residual cartilage apparently develops from the dormant cartilage rim which surrounds the f.a.f In most cases this new cartilage occurs only in part of the f.a.f (Fig. 3) while in other cases it occupies the entire fissula as a ch ndromatous mass (Fig. 4) Most often these changes are encountered (1) along the cochlear margin of the f.a.f (2) above and at the tympanic opening, (3) between the tympanic opening and the stapedial joint, and (4) at the vestibular end (Fig. 3) The changes set in at a late stage of foetal life and are common in newborn and children (Bast, 1936)



Fig 5 Phot micrograph showing sclerotic bone tissue (*SB*) i the fissula at fenestram in 31 year-old woman. Th well-defined, lamellated (deep idophilic in H E staini g) new bone with a fi reticula net is occup ing the entire fissula. Intrach ndral bone is visible also. 40.

This cartilage has been called abnormal, as it differs from the cartilage rim which normally surrounds the *f.a.f* in showing marked activity (Fig 4) or large lacunae with calcified matrix indicating incipient ossification. In haematoxylin-eosin-stained preparations this is characterized by the deep staining of the matrix. Most of these chondromas appear in large fissulae where the histological activity is believed to be pronounced in an attempt at narrowing the wide fissula.

Normally (Fig 2) there is gradual transition from perichondral connec tive tissue, by way of new formed cartilage, to the surrounding intra-endochondral bony tissue, but there is a sharp borderline between the condromatous cartilaginous masses and the intra-endochondral bony tissue (Fig. 4)

The formation of the neoplastic bony mass (sclerotic bone) takes place at a time when the ossification of the foetal capsular cartilage ought to have been completed Therefore the ossification becomes either intrachondral or osteoid, depending upon the time at which the active cartilage undergoes ossification (Bast & Anson, 1949)

As emphasized by Anson *et al* (1948) the new formation of sclerotic tissue is distinctly visible in the histological sections (Fig 5) The new formed bony tissue stains deeply acidophilic by haematoxylin-eosin showing a sharp demarcation from the surrounding endochondral bony tissue It may

wholly or partially obliterate the f.a.f It is made up of well-defined lamellar bony tissue arranged around distinct vascular cavities in which there is a delicate reticular net. The central cavities are larger than Haversian canals in normal tissue.

This bony tissue invades the surrounding bony tissue unlike the above mentioned chondromatous masses. This invasion is said to involve the anatomical structures in this region, in particular the borders of the oval window including the foot plate of the stapes, resulting in ankylosis of the joint and consequent clinical otosclerosis.

MATERIAL

Our material consists of 453 human temporal bones from various age groups without otosclerosis, distributed as follows

(1) 11 temporal-bone primordia from nine foetuses, varying in C–R length from 56 to 123 mm, representing the period 3rd–5th foetal month. In weight the foetuses ranged from 11 g to 109 g

(2) 140 temporal bones from 75 newborn infants, ranging in weight from 800–3900 g, corresponding to birth in the 7th–10th foetal month. Three of the infants had been stillborn, while 07 lived for a maximum of 3 days, 14 for 21 days, and 1 for 3 months.

(3) 26 temporal bones from children aged 1–12 years.

(4) 276 temporal bones from 174 adults aged 13–93 years.

HISTOLOGICAL TECHNIQUE

The temporal bones were fixed in 10% neutral formalin, decalcified in a buffer solution of formic acid and sodium formiate (Kristensen, 1949) and embedded in celloidin. The prepared blocks were cut on a special microtome into sections of approx. 18 μ All the preparations were cut in the horizontal plane from the superior semicircular canal to the level of the cochlear aqueduct. Every 10th section was stained with haematoxylin-eosin. The remaining sections were stored

OBSERVATIONS

Our findings in foetuses, newborns, and children are given in Table 1

Foetuses

Our youngest foetus measured 56 mm in C–R length, corresponding to the 12th week. In this case the f.a.f primordium was discernible only at the vestibular end. At this site the cells in the precartilaginous rim had undergone a change from round to more spindle-shaped cells. At the same time vacuolation of the tissue had started. The relation to the vestibular periotic tissue was clear

TABLE 1 *Histopathological findings in the fissular region of 177 temporal bones from foetuses, newborn and children*

	Age	Fissula open			Fissula blocked	
		Normal	Secondary new formation of cartilage or bone in the f.a.f. also and on a level with the tympanic opening and between the f.a.f and the cochlea	New formation of bone round the f.a.f from the tympanic opening to the stapes	Secondary cartilage in the tympanic opening	Ossified cartilage at the vestibular end of the f.a.f
Foetuses	3rd–5th month	11				
Newborn	7th–10th month	19	86	8	25	2
Children	1–12 years	6	10		10	
Total		36	96	8	35	2
Percentage			79		21	

Our other specimens from foetuses and newborns showed the continued foetal development of the fissula, except for the period 5th–7th foetal month which is not represented in our material. The development is characterized in particular by the conversion of connective tissue into cartilaginous tissue in the fissular periphery and by the central connective tissue acquiring a more areolar arrangement and showing a fine reticular connective tissue with small blood vessels (Fig. 1 *c*).

Newborns and infants

In the greater part of the temporal bones from newborns and infants (79 %) the f.a.f. was found to be open, i.e. it had a tympanic opening communicating with the vestibular opening. In the remaining temporal bones (21 %) *the tympanic opening was blocked by cartilage and/or new formed* bone. Only two bones showed the vestibular opening to be blocked by ossified cartilage.

Among the newborns and children we found 25 temporal bones (15 %) to be "normal" (Fig. 2), i.e. with an open fissula without secondary cartilage formation. In 141 of the temporal bones in this group (85 %) secondary cartilage formation had taken place in the fissular region (Figs. 3 and 4) of a localization as apparent from Table 1. These were the above-mentioned cartilaginous formations and the larger chondromatous masses. None of these temporal bones showed new formation of sclerosed bony tissue.

Adults

The f.a.f. was discernible in all our adult temporal bones. The findings are given in Table 2.

TABLE 2 *Histopathological findings in the fissular region in 9 6 temporal bones from adults.*

Normal	1 6
Sec. cartilage	53
Sclerotic bone	45
Vestibular end blocked	
Tympanic end open	35
Preotosclerotic foci	32

TABLE 3 *Correlation between the histopathological findings in the fissular region of 166 temporal bones from newborn and children and of 2 6 temporal bones from adults.*

	Newborn and children (166).	Adults ([illegible]).
Normal	15	64
Sec. cartilage – chondromas	85	20
Sclerotic bone	—	16
Preotosclerotic foci"	—	32 (12 %)

In 176 temporal bones (64 %) we have interpreted the findings as "normal". The histological structure of the f.a.f. showed, as mentioned above, loose connective tissue centrally surrounded by a cartilaginous rim separating it from the osseous labyrinth.

In 53 cases (20 %) dormant cartilage had given rise to secondary cartilage formation resulting in well-defined cartilaginous masses. In 45 cases (16 %) sclerosing had taken place, the f.a.f. being wholly or partially obliterated by new-formed bony tissue with large medullary cavities (Fig. 5).

In 32 cases (12 %) we found in the fissular region bony changes which in our opinion may be interpreted as incipient otosclerosis. As shown in Fig. 6 these are changes of the labyrinthine capsule localized in close relation to the f.a.f., as a rule just beneath the periosteum of the promontory between the oval window and the cochleariform process. Out of the 32 cases showing "pre-otosclerotic" foci, 10 exhibited sclerosing at the site of the f.a.f., secondary cartilage formation in 3 cases and a normal fissula in 19. Table 3 shows the correlation between the histopathological findings in the fissular region from newborn, children and adults.

DISCUSSION AND CONCLUSION

There is hardly any region of the labyrinthine capsule which exhibits such varying histological structure as the fissular region or where processes of repair and/or destruction may occur throughout life. It is not yet known

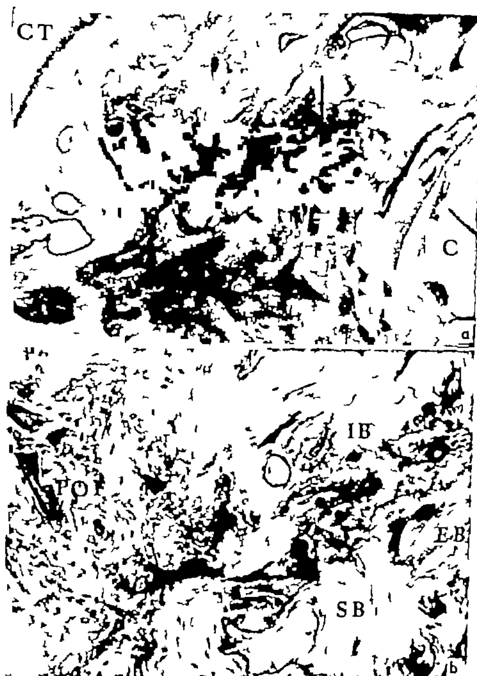


Fig 6 Phot m crograph from 28-yea -old woman showing i the same temporal bone scleroti bone t su (*SB*) occupying the fissula ant fenestram nd "preot sclerotic" focu (*POF*) f subperiosteal situation beneath th cochleariform process. I trabondral bone i present lso. (*b*) shows the difference between the two tissues i the rea indicated i ( ) ( ) 25, (*b*) 36.

such pathological changes of the f.a.f. can spread to the neighbouring oval window and the foot plate of the stapes, compromising their function.

Anson, Bast, and their co-workers pointed out that the named secondary cartilaginous new formations and the subsequent ossification of the tissue forms the basis for the development of otosclerotic tissue. This would then have to extend beyond the fissula, reaching the oval window. As pointed out by themselves (Anson & Bast, 1949, p. 278), however, they still have to supplement their studies by a sufficient number of temporal bones from older children and from adults to be sure that the above mentioned changes do take place.

Their theory has in fact been undisputed ever since. Only Guild (1944, 1950) has objected, being unable to accept it on the basis of his studies on the histology of the otosclerotic focus.

As a rule, the secondary new formation of cartilage has been referred to as "abnormal". It is true that the new formation of cartilage is apparently of a character differing from normal. But this also seems to be the only "abnormal" feature, as these formations are common. In our studies they were present in a high percentage, viz. in 85% of the newborns and children (141 out of 166 temporal bones) and in 20% of the adults (54 out of 276 temporal bones). In the adults the secondary cartilage remnants have apparently become quiescent, or at least they have not developed further into sclerosis.

If it were right that otosclerotic bony tissue arises from a secondary new-formation of cartilage, all similar cartilaginous nodules would be expected to result in the formation of otosclerotic bony tissue. However, Bast (1936) himself found that out of 52 temporal bones from foetuses and children up to 6 years of age 42 contained cartilaginous or osseous masses in the fissular region. In 1948 it was reported by Anson *et al.* (1948) that in 16 temporal bones from 27 foetuses and children up to 10 years they had found cartilaginous masses in the f.a.f.

Anson & Bast (1949) concluded that since this percentage is far too high in relation to clinical experience as to the incidence of otosclerosis, "other types of histological succession may occur". They believed that neoplastic new-formation of bone (sclerosing) was rare (2–3%) and that perhaps only the chondromatous masses ended as otosclerotic tissue.

Among 276 temporal bones from adults we found 45 (16%) to show distinct new-formation of sclerotic bone in the f.a.f. as stated above. This bony tissue is of a histological appearance entirely different from that of otosclerotic tissue. This is apparent from Fig. 7 which f.a.f. is partially obliterated by sclerotic bony tissue while simultaneously a typical otosclerotic focus may be seen outside the fissula ante fenestram. The same phenomenon is illustrated by Fig. 6 in which the secondary cartilage formations are seen together with new formed sclerotic bony tissue in the f.a.f., while the region towards the tympanic cavity shows tissue which we interpret as a small otosclerotic focus ("preotosclerosis") (Jørgensen & Kristensen, 1967).



Fig 6 Phot micrograph from 28-year-old woman showing i the same temporal bone sclerotic bone tissu (*SB*) occupyi g the fissula ant fenestram nd "preotosclerotic" focus (*POF*) f subperiosteal situation beneath the cochleariform process. Intrachondral bone i prese t lso. (*b*) hows the difference between the tw tissues i the rea indicated in (*a*) (*a*) 25, (*b*) 38

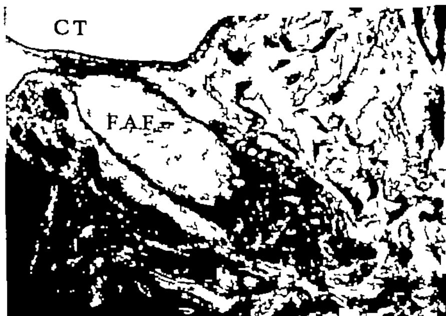


Fig 8 Photomicrograph showing an otosclerotic focu (*OF*) localized t the bottom of the fissul nt fenestram. From 84-yea -old man. 40.

In other words, we do not think that the named sclerosing of the second ary cartilage in the f.a.f is invariably a precoursor of the otosclerotic focus. We do not deny that an otosclerotic focus may well arise in the f.a.f As exemplified in Fig. 8 otosclerotic bony changes may be present in the f.a.f without this section, or nearby ones, showing any trace of secondary cartilage formation or sclerosing of the bone The histological architecture of the tissue in this case corresponds accurately to that of an otosclerotic focus.

Although it has been shown by clinical and histological experience that the majority of otosclerotic foci are localized in the antefenestral region (80–90%) we believe that in general a focus does not arise in or from the fissula ante fenestram. Out of the 32 cases in which we found "preotosclerotic" foci the majority were localized in the subperiosteal layer beneath the coch leariform process outside the fissula ante fenestram. Indeed, it is well known that otosclerotic foci may arise in other sites of the labyrinthine capsule without any relation to the above-menti ned sites where residual cartilages may be found (Guild, 1944 Bretlau 1969)

ZUSAMMENFASSUNG

Die Struktur und das hist l gisch Bild der Region der Fissula ante fenestram in der Labyrinthkapsel wurden in 453 Schläfenbeinen von Embryonen, Neug borenen, Ki dern und Erwa hsenen untersucht. Unsere Funde unterscheiden sich n früheren U tersuchungen über der Fissula ant fenestram (Bast, Anson, Will-

son und Mitarbeiter). Wir meinen nicht, dass die sekundäre Knorpel- und Knochenbildung in der Fissula ante fenestram immer in einer Bildung der otosklerotischen Herde resultieren. Der Unterschied zwischen diesen Geweben wird diskutiert und mit histologischen Funden beleuchtet.

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OXYGEN CONSUMPTION OF THE MEMBRANOUS COCHLEA AND OTHER TISSUES IN SHAKER 1 (sh 1/sh 1) AND NORMAL (CBA-J/CBA-J) MICE

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The o ygen consumpti n on the membranous cochlea of the sh-1/sh-1 was significantly less than that of the CBA J/CBA J Other tissues showed no difference in their oxygen consumption.

Shaker 1 (sh 1/sh 1) mice are a strain of mice with a progressive deafness which is inherited as an autosomal recessive. The gene for shaker 1 is located on the first linkage group (Sidman *et al.*, 1965) There have been several studies in which the physiology (Mikaelian & Ruben, 1964 Brown & Ruben, 1969) behavior (Hack, 1968) histology (Gruneberg *et al* 1940 Deol, 1956) and electron microscopy (Kikuchi & Hilding, 1965 and 1967) of the sh 1/sh-1 have been described. The present study is an examination of the ability of the membranous cochlea of the deaf sh-1/sh-1 to utilize oxygen. Other tissues were examined to determine if the shaker 1 gene affected them in a similar fashion The CBA-J/CBA J mouse was used as a comparison as some of the other studies done with the sh 1/sh-1 also used this method.

METHOD

The oxygen consumption was measured by means of the Warburg con stant volume manometer (Umbreit *et al* 1964) Fifty sh-1/sh 1 and 50 CBA-J/CBA-J mice were used. All of the sh-1/sh 1 animals were behaviorally deaf as tested by the Pryer reflex.

The animals were sacrificed by cervical dislocation, the temporal bones were immediately removed and the bullae were opened to expose the cochleae. The dissecting microscope was used to remove the bony capsule of the cochlea, the surrounding connective tissue and the nerve tissue within the modiolus. The remaining membran us cochlea was placed in 0 1 *M* phos-

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phate buffer pH 7 0 at 0 C. The specimen consisted of the membranous cochlea, the modiolus and the osseous spiral lamina. Preliminary experiments showed that the modiolus and osseous spiral lamina caused not detectable changes in the manometric readings which indicated that the oxygen consumption of these tissues was negligible This finding was in agreement with previous studies (Oda, 1963 Sato *et al* 1968)

The dry weight of the preparations of CBA-J/CBA-J and sh 1/sh-1 cochleae showed no significant differences. Thus, any differences in oxygen consumption per unit weight would be due to differences in the ability of the membranous cochlea to utilize oxygen.

Each determination of oxygen consumption was made by homogenizing 10 membranous labyrinths of either sh-1/sh 1 or CBA-J/CBA-J in 5 0 ml of 0 1 *M* phosphate buffer (pH 7 0) at 0 C using Potter s microglas homogenizer Other studies (Oda, 1963 El Mofty & El Serafy 1966) of oxygen consumption have used Krebs-Ringer s phosphate buffer as the medium. Preliminary experiments for the present study revealed the oxygen consumption was 50% greater using the Krebs-Ringer s phosphate buffer (pH 7.2) but the variability between each sample was large

Five separate determinations were made for sh 1/sh 1 and for CBA-J/CBA-J Determination numbers 56, 57 and 58 consisted of membranous cochleae of sh-1/sh-1 and CBA-J/CBA-J one month of age, and determination numbers 59 and 60 consisted of membranous cochleae from sh 1/sh 1 and CBA-J/CBA-J one year or older The determinations of the oxygen consumption for brain, liver kidney cortex and femoral muscle were made by removing 0.5 gm of tissue from one animal. The tissue was homogenized in 1 0 ml of 0 1 phosphate buffer (pH 7 0) at 0 C. Five determinations were made for the sh-1/sh 1 brain 3 determinations were made for sh-1/sh-1 kidney cortex 3 for sh 1/sh-1 liver and 3 for sh 1/sh 1 femoral muscle An identical number of determinations were made for the tissues of the CBA-J/CBA-J mice Each homogenate was placed in a manometric flask which contained 0.5 ml of distilled water The side flask contained 0.5 ml, 0 1 *M* succinate as the substrate. The temperature of the water bath was kept constant at 37 C and the shaking speed was 60 oscillations per minute with a stroke amplitude of 4 cm Measurements were made every 10 minutes for one hour Oxygen consumption was expressed as microliters of oxygen/homogenate/unit of time

RESULTS

The mean oxygen consumption for the CBA-J/CBA-J cochleae at the end of one hour was 2 14 $\mu l\ O_2$/homogenate/hour and that of the sh 1/sh-1 membranous cochleae was 1.32 $\mu l\ O_2$/homogenate/hour (Fig 1) The observed difference between the two was tested with the *t* test and was significant to less than 1% There was no demonstrable difference between the one-month-old and year-old cochleae The oxygen consumption for the brain,

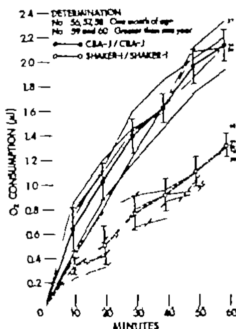


Fig. 1

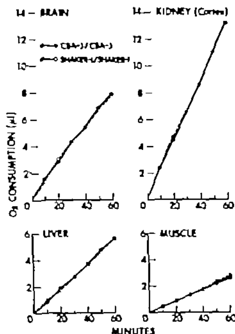


Fig. 2.

Fig. 1. Compariso f oxygen consumption f the membranou cochlea of sh-1/sh-1 & CBA-J/CBA J Vertical lines equal tw standard devi tions.

Fig. 2. Oxyge consumption f brain, kidney li er & m scle f CBA-J CBA-J & sh-1/sh-1

liver kidney cortex and femoral muscle for the CBA-J/CBA-J and sh-1/sh-1 was not significantly different (Fig 2)

DISCUSSION

There was a significant difference in the amount of oxygen consumption of the membranous cochlea between the sh-1/sh-1 and CBA-J/CBA-J with the oxygen consumption of the sh-1/sh-1 being less than that of the CBA-J/CBA-J These results indicate that there is a defect within the membranous cochlea of the sh 1/sh 1 mouse which results in a low level of oxygen con sumption. The possibility that the difference in oxygen consumption could come about because cells of the membranous cochlea of the sh-1/sh 1 were replaced by other cells was controlled by using the one month-old sh-1/sh 1 in which there is no hist logical demonstrable differences in the cochleae (Mikaelian & Ruben, 1964)

The dimunition of oxygen con umption was confined to the membranous cochlea and wa not found in other tissues. The action of the shaker 1 gene is probably not directly on the respiratory enzyme system, as the other ti sues would have shown a defect because each cell of the membranous

cochlea, brain, liver kidney and muscle of the homozygotic shaker 1 has both sh 1 genes. A simple hypothesis which can account for this is that the decrease in the oxygen consumption is due to an abnormal product which is synthesized by the affected cells in the membranous labyrinth and that this abnormal product is not synthesized by the other cells in the body This is a reasonable hypothesis as highly differentiated cells are known to manufac ture unique products.

RÉSUMÉ

La dépense d oxygène du limaçon membraneux des souris expérimentales (sh 1/ sh 1) était moins importante que celles des souris normales (CBA J/CBA J) Il n y avait pas de différence de la dépense d oxygene dans d autres tissues.

ZUSAMMENFASSUNG

Der Sauerstoffverbrauch der Membranen-Cochlea „sh-1 sh-1" war erheblich geringer als der der CBA-J/CBA J Andere Gewebe zeigten keinen Unterschied im Sauerstoffverbrauch.

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THE GROWTH OF THE OTIC CAVITIES IN THE HUMAN FOETUS

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The cavitation f the early oti capsule was studied in a material of 16 human foetuses ranging from 12–125 mm crown–rump length. It was found that synchronously with the vascularization of the periotic tissue new type of cell appears. This cell is identifi d as a histiocyte with the ability to chondrolyse. The present work could not confirm previous studies reporting th formation f precartilage and cartilage in the periotic tissue and subsequent dedifferentiation to co nective tissue.

A recent study (Andersen & Matthiessen, 1966) on the histiocytes of the human foetus showed histiocytes that are involved both in the breaking down of cartilage matrix during the invasion of vascular mesenchyme in the epiphyses of the cartilages and in the resorption of bone and calcified cartilage during the invasion of the periosteal tap

The purpose of the present study was to demonstrate a similar mechanism involved in the cavitation of the otic cartilage and bone of the human foetus.

MATERIAL AND METHODS

The material comprises 16 human foetuses removed by Caesarian section from the uterine cavity in connection with legal abortion On the basis of crown–rump length (c.r l.) the material is distributed between 12–125 mm, all measurements being made on the unfixed foetuses.

Fixation

Prior to fixation the otic capsule was removed and divided into conveni ently thin sections to facilitate the penetration of the fixatives.

The temporal bone of the one side was intended for enzyme cytochemical studies and fixed in ice-cold formolcalcium (Pearse, 1960) for 6–24 hr The tissue was then transferred to two shifts of gumarabic-sucrose (ice-cold) The other temporal bone was fixed in Lillie s ethanol–formalin–acetic acid (ice-cold) (Lillie 1954) for 48 hr both for morphological studies and for demonstrating acid mucopolysaccharides and glycogen

After fixation the tissue for enzyme histochemical studies was cut into 6–8 μ sections on a Jung "Frigotome with a cryostat chamber and anti-roll device or in a Pearse-Slee cryostat (type HR)

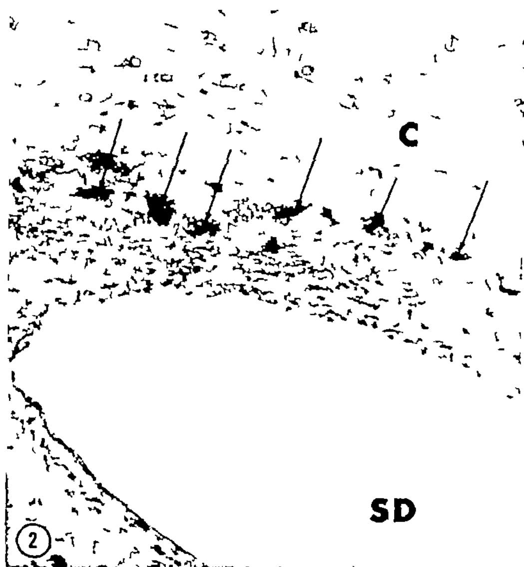


Fig 2 Higher magnificati n f th pper gro p f histiocytes (arrows) from Fig. 1. (C) Cartilage; (SD) semicircul duct. N phthol-AS-BI-phosphat method for cid phosphat se 160

time liberation of chondrocytes from the opening of cartilage cell lacunae is observed Cellular degeneration or phagocytizing of chondrocytes are not seen as is the case in the metaphysis of the anlage of the long bones in the extremities (Andersen & Matthiessen, 1966)

The same is observed in the canals carrying vessels and nerves.

DISCUSSION AND CONCLUSION

The above-mentioned cells show the same morphology and cytochemistry as phagocytizing cells recently described by Andersen & Matthiessen (1966) and Andersen (1968) The authors considered these to be histiocytes origi



Fig. 3. Section showing histiocytes (arrows) in close contact with the cartilage and containing small round, metachromatic cytoplasmic granules. From the periotic mesenchyme surrounding the vestibule. Toluidine blue. ×400.

nating from primitive leucocytes emigrating from the vessels. The nodular cytoplasmic localization of the final reaction products of acid phosphatase and unspecific AS-esterase indicates that the cells contain lysosomes. These enzymes are such common constituents of lysosomes that they are considered to be the most important characteristics in the cytochemical detection of lysosomes. This is further supported by the presence of the small PAS-positive granules in the cytoplasm of the cells. It is well known that both the lysosomes and the phagolysosomes are surrounded by a lipoprotein membrane.

No doubt the cells exert a certain pinocytotic or phagocytotic activity

(Andersen & Matthiessen 1966) This is supported by the accumulation in the cells of hyaluronidase-digestible metachromatic or Alcian blue stained material which is presumably digested cartilage matrix. The chondroclastic activity is furthermore indicated by the appearance of groups of *these cells in close contact with the surrounding otic cartilage just where* cavitation develops

Early in the development there are large quantities of these cells apparent in the periotic tissue where no multinucleated chondroclasts are seen.

In accordance with the ability of the cell to chondrolyse and osteolyse (Andersen & Matthiessen, 1966 Andersen, 1968) it is justified to consider these cells responsible for the continuous cavitation of the different canals and cavities of the inner ear

A recent electron microscopical study (Anderson & Parker 1966) stated that vascular invasion may give rise to chondrolysis However in the *present study blood vessels were never seen in close contact with the cartilage*

During the later stage of development when an otic bone capsule has developed, the multinucleated osteoclasts presumably originate from the histiocytes by fusion (Andersen & Matthiessen 1966 Andersen, 1968)

Previous authors (Streeter 1918 Bast & Anson, 1949) have claimed that immediately after the ducts are formed the surrounding connective tissue changes into a precartilage *which during the eighth week has matured into* true cartilage According to Streeter the growth and expansion of the canal spaces are accomplished by a retrogressive differentiation of the cartilage

The present study is in disagreement with the above-mentioned papers, since no signs of formation or dedifferentiation of precartilage could be demonstrated in the periotic tissue From the very beginning, the chondrogenous tissue and the periotic tissue are dense and avascular The latter will soon be *vascularized and transformed to looser tissue with reduced inter* cellular metachromasia and stainability with Alcian blue This is in accordance with the *development* of the interzone of the joints (Andersen 1962 *a* and *b* 1963, 1964 1968) of the eyelids (Andersen *et al.*, 1965) of the enamel reticulum (Matthiessen 1963) of the palatine processes (Andersen & Matthiessen, 1968) and of the villi of the small intestine (Garbarsch, 1968) Presumably it is due to hydration of the acid mucopolysaccharides in the form of an osmotic effect (Mathews, 1967)

It would be of great interest if remaining histiocytes could be found in the otic capsule in later life and eventually take part in the remodelling processes of this bone under normal and pathological conditions. Many aspects of such processes are so far unexplained.

ZUSAMMENFASSUNG

Die Kavernenbildung der Labyrinthhohlräume wurde in einem Material von 16 Menschenembryonen kranio-kaudale Länge 12 125 mm, untersucht. Es wurde gefunden, dass gleich zeitig mit Einwandern von Gefässprossen in den Labyrinth-

knorpel ein neuer Zellentyp erscheint. Diese Zelle ist als eine Histiozyte mit chondrolytischem Vermögen identifiziert worden. Die vorliegende Arbeit konnt nicht frühere Studien bestätigen, die die Bildung on Labyrinthhohlräumen als el e Dedifferenzierung früher gebildeter Knorpel ansahen.

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STATISTICAL EVALUATION OF HEARING LOSSES IN MILITARY PILOTS

(2nd Report)

G v SCHULTHESS

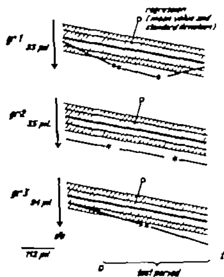
From the Aeromedical Institute of the Swiss Air Force Dübendorf Switzerland

The dependance of the evolution of the hearing loss of military pilots on age and a measurable amount of flight noise exposure can be represented as a function calculated by the technique called regression. A certain number of subjects from a larger test group manifests a hearing loss which exceeds the calculated norms to a highly significant degree. Most of these pilots have undergone an additional noise exposure which explains the deviation from the standard group These findings support the assumption that in addition to the direct or indirect consequences of ageing, daily noise exposure tends to contribute to the development of presbyacusis resulting in an extraordinarily rapid increase of hearing loss in comparison with age

In a previous report (v Schulthess & Huelsen, 1968) we calculated the rate of increase of the hearing loss of 6/3 military pilots in relation to their ages and to their exposure to flight noise Threshold audiometrical tests collected from 1950 to 1967 served as the basis of this survey The threshold shifts for the frequencies 1000 2000 4000 and 8000 cps were computed and the progression of the hearing loss could be represented as a function of age and exposure to flight noise These functions were derived by applying regression analysis to the data

The *present study* deals with the frequencies 2000 4000 and 8000 cps which are known to be most affected by presbyacusis and acoustic trauma. For these frequencies, larger regression coefficients were observed, thus demonstrating a more rapid increase of the threshold shift with age than in lower frequences.

Among the 6 3 pilots included in our study we found a number f 112 whose hearing loss for one two or all of the mentioned test frequencies once or repeatedly exceeded the mean value by at least 1 96 times the standard deviation, which statistically is of high significance (Fig 1) These subjects were selected for further study It could be assumed that they had suffered from an either temporary or permanent threshold shift in addition to the expected hearing loss for the corresponding age group. The members of the selected group were checked clinically in search for the reasons for



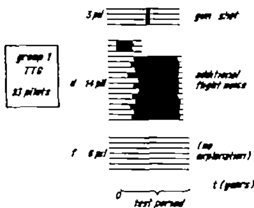


Fig 1. | Fig 2.

Fig 1 E l tion f hearing loss (threshold shift) Mea value and standard deviati n. Beha iour of additional hearing loss (———) schematically

Fig 2 Group 1 Reasons f additional temporary heari g loss nd occurrence i rel tion t test period.

the abnormal evolution of their hearing loss. Each pilot has a detailed medical dossier which furnishes complete information on occasional viral or bacterial infections, acute or chronic diseases and on focal, rheumatic, metabolic or endocrinological conditions which might influence hearing In view of the fact that the cochlea may be sensitized to noise damage even by subtoxic application of ototoxic medicaments (Podvinec & Stefanovic, 1966 v Schulthess, 1961) special attention was focused on any history of streptomycin or quinine medication Cases of perceptive deafness in the pilot s family and additional noise influence were also looked for

In our test groups an abnormal increase of the threshold shift ("additional threshold shift) manifested itself in three ways represented schematically in Fig 1

Group 1 (23 cases) The additional hearing loss is transitional and thus represents a temporary threshold shift (TTS)

Group 2 (35 cases) The additional hearing loss preexisted, when the pilot entered the test period (permanent threshold shift)

Group 3 (54 cases) The additional hearing loss increased suddenly or continuously during the test period (perman nt threshold shift)

The dossiers of our 112 subjects g e the following data in Figs. 2–4 the indices *a* to *f* are used uniformly *a* ( ) = gun-shot trauma *b* ( ) = blow to the head *c* = industrial noise *d* = additional flight noise exposure from pro-

The data ha been collected by G G isard.

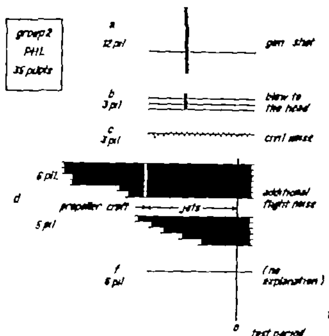


Fig 3 Group 2 Reasons f r dditional permanent hearl g los equired before enteri g test period.

peller craft and/or jets e = operative inner ear damage f = no relevant data)

Group 1 (Fig 2) The additional hearing loss in these 23 pilots has a *temporary* character Therefore, these are possibly cases of temporary threshold shift due to auditory fatigue or spurious variation in singular audiometric tests.

(*a*) Three pilots had a TTS due to gun shot trauma

(*d*) In two pilots the audiometric measurement was taken in the course of the first flight training period when the exposure to flight noise is more than usual 14 subjects were either full-time test pilots, instruction or maintenance personnel who were more exposed to flight noise than were civilians. (In Switzerland the officers of the air force are mostly civilians who do their military duties in terms of several weeks each year)

(*f*) In six persons no reason for the additional hearing deficiency could be found.

Group 2 (Fig. 3) 35 pilots suffered from an extraordinary additional hearing loss when entering the test period

(*a* + *b*) In 15 subjects this was found to be the consequence of a gun shot trauma or a blow to the head

(*c*) Three pilots had civil occupations in a damaging noise environment

(*d*) Six older officers (instructing staff) had begun their flying career on propeller craft with non isolated cabins and automatic weapons aboard. The

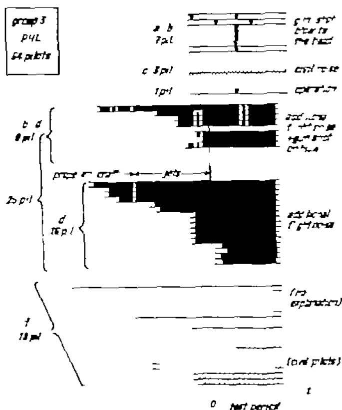


Fig. 4. Group 3. Reasons for additional permanent hearing loss manifesting itself during test period.

deleterious effect of such conditions on hearing is known. Five other pilots (instructing staff) had their flight training and additional noise exposures several years before entering the test period.

(*f*) For six pilots no apparent exogenous explanation for the abnormal pattern in the evolution of the hearing loss was found.

Group 3 (Fig. 4). 54 pilots suffered an additional permanent threshold shift or showed an extremely rapid evolution of their hearing loss during the test period.

($a+b$) In seven cases one or several acute injuries (gun-shot, blow to the head) caused the permanent hearing loss.

(*c*) Three pilots were employed in an environment with damaging noise.

(d) Twenty five pilots of this group are full-time pilots with an additional flight-noise exposure. Nine of these ($d+a+b$) had supplementary acute injuries.

(*e*) In one case a middle ear operation led to the inner-ear lesion.

(*f*) In eighteen subjects no reason could be found. However, eight of these were civil air-line pilots whose exposure to noise may be higher than usual

In the dossiers of the selected group no other etiologic, pathogenic or potentially sensitizing factor could be found in such an important number as to attribute any effect on hearing to it Nevertheless the heredity of inner ear deafness has to enter into discussion The number of cases with a family history of a sensori neural hearing loss was relatively high in group 3 (11 cases) as compared with groups 1 and 2 (together 6 cases) and with the total of 673 pilots (112 cases)

COMMENT

As pointed out in a former paper the increase of high tone loss depending on age and a certain measurable amount of noise exposure can be demonstrated graphically The regression coefficient gives the slope of the curve showing this relationship and thus represents the rapidity of the diminution of hearing for a given frequency with time The relationships were calculated for a rather homogeneous group of 673 military pilots. Of these 112 were found to differ in a highly significant manner from what would have been expected by statistical calculation. Thus the problem arose what could be the reason for the abnormal behaviour of the hearing in the group To solve this problem, the medical and military dossiers of each pilot of the selected group were checked for any kind of influence which could cause the additional hearing loss. In 75% (78 pilots) an *additional exposure* to severe noise (gun shot blow to the head, industrial and flight noise) was found to give the most plausible explanation Endogenous factors as postulated by Davis *et al* (1950) and Glorig *et al* (1958) which could have favoured the establishment of a permanent threshold shift or the development of a temporary threshold shift were not found Variations in audiometric technique may be important but only in group 1 with the temporary threshold shift In groups 2 and 3 the permanent deviation from the standard values has been confirmed several times at intervals of some weeks or even months. Heredity may have influenced the evolution of the hearing loss to a certain degree

Our findings appear to be consistent with the general experience and bring forward the following points for discussion

(1) The most evident feature of our analysis is the dependance of the hearing loss on age the different frequencies being involved in a diminishing degree from the higher to the lower ones.

(2) A measurable amount of noise influence of a certain duration and specified quality (average flight noise) during military services tends to increase the average hearing loss.

(3) Individual pilots whose hearing loss seems to increase significantly more rapidly than would be expected in a great number of cases have suffered additional noise influence This fact seems to confirm the importance of the life long influence of various noise of a certain intensity on the hearing acuity at threshold (Gravendeel & Plomp, 1960 Jatho & Heck, 1959)

(4) In our test group no endogenous eventually sensitizing factors, with the possible exception of heredity seemed to contribute to the appearance of a temporary threshold shift or the establishment of a permanent hearing loss.

CONCLUSIONS

The ageing of the sense of hearing, so-called presbyacusis, is directly or indirectly the consequence of over-all ageing of the human organism This endogenous process is very complex and the sense organ itself as well as the VIII nerve and the central pathways, seem to be involved (Schuknecht, 1964) According to our experience there is evidence too that the exposure to more or less damaging noise in daily life contributes to the clinical entity of presbyacusis. Audiologically the endogenous and the exogenous factors of presbyacusis are not separable. We do not discount the idea that there may be individual variations in the resistance of the sense of hearing to exogenous noise influence in daily life The character of this variation remains un known but may be hereditary

RESUMÉ

Chez u groupe d aviateurs militaires l'évol tion de la perte d ouïe sur les fréquences aigues a été calculée par la méthode statistique dite analyse de régression. Ainsi la dépendance de la diminution uditive de l'âge du sujet et du bruit de vol habituel uquel celui avait été exposé, pu être déterminée. Nous avons sélectionné 112 (du total d 673) pilotes dont les seuils auditifs différaient d un manière significative des régressions calculées. La plupart d ces individus se montraient d'avoir été exposé à des bruits ultérieurs. Ain i nous pensons qu une surdité due aux bruits de la ci ilisation fait parti du phénomène d la surdité progressive des agés d te presbyacousie dont l'étiologie et la pathogénie sont connues d'être très comple es.

ZUSAMMENFASSUNG

Vermittels Regressionsanalysen wurde die Progredienz des schwellenaudiometrischen Hoeht n erlustes von 673 Militärpiloten mit bekannter Fluglärmex position rechnerisch ermitt lt. 112 Piloten, welche um den 1,96f chen Streubereich über dem ermittelten mittleren Hörverlust liegen, wurden hera sg griffen und im Hinblick auf ev Ursachen ihres aussergewöhnlichen Verhaltens untersucht. In der Mehrzahl der Fälle ko nten zusätzliche Lärmbelastungen als wahrscheinlich Ursache ermitt lt werden. Damit glauben wir n hmen zu dürfen, dass ein belastungsbed ngt Abnützung des Perzepti nsorgans und seiner nervösen Elements als Teilursache der Presbyakusi angesehen werden muss.

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FIBROUS DYSPLASIA OF JAW BONES

A Clinical Roentgenographic and Histopathologic Study

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A clinical, roentgenographic and histopathologic study of 20 cases of fibrous dysplasia of jaw bones is presented. The ratio of localization to th upper and the lower jaw was 16 4, and the ratio of men to women 8 12. Roentgenograms and histopathologic pictures of cases observed over several years are shown. Roentgenographically diagnosis is most difficult to establish at early st ges of development and must be verified by histologic examination Since fibrous dysplasia f jaw bones is a benign lesion and becomes gradually stationary the proper therapy fo th conditi n is local "cosmetic resection. Radical surgery is not indi cated in cases with large extension.

Fibrous dysplasia of jaw bones is a fairly rare disease. Its macroscopic, as well as its microscopic picture, can vary considerably from one case to the other depending on the degree of maturity of the pathologic process. For this reason, it has been described under various names, such as fibrous osteoma of the jaws, ossifying fibroma (Waldron, 1953 Boyd, 1961) and osteoma (Gold, 1955) Some clinicians and pathologists still use other designations for the same condition central osteoma, fibro-osteoma, hyper trophic localized osteitis and localized osteitis fibrosa (Ringertz, 1938 Bill ing & Ringertz, 1946 Gold, 1955) Such a loose terminology leads to discrepancies in diagnosis, and also difficulties in making decisions on therapy Several authors (Pugh, 1945 Schlumberger 1946 Jaffe 1953, 1961) have stated that fibrous dysplasia of jaw bones should be the designation for this condition A strong argument in favour of this term is the fact that localization is the only difference between these lesions and fibrous dysplasia of any other skeletal region (Schlumberger 1946 Jaffe, 1953 Berger & Jaffe, 1953)

The etiology of fibrous dysplasia is unknown. Most authors (Billing & Ringertz, 1946 Jaffe 1953, 1961 Berger & Jaffe 1953 Boyd 1961) regard it as a developmental defect, and some (Ringertz, 1938 Waldron, 1953

TABLE 1 *Localization and sex distribution in earlier published cases of fibrous dysplasia of the jaw bones*

Author	No. of cases	Maxilla	Mandible	♂	♀
Billig & Hüggerts (1940)	13	8	5		8
Schlumberger (1946)	9	7	2		
Schlumberger (1946)	12	7	5		
Waldron (1953)	2		2	1	1
Houston (1965)	8	3	5	3	5

Thoma & Goldman 1960) are of the opinion that fibrous dysplasia is a neoplasia. Trauma as an etiological factor is mentioned (Schlumberger 1946)

In the majority of previously published papers (Table 1) fibrous dysplasia was more frequent in the upper jaw than in the lower and had a higher incidence in women than in men Since clinical symptoms of fibrous dysplasia of jaw bones are vague and uncharacteristic one is dependent on roentgenographic and especially on histopathologic findings (Houston 1965)

The object of the present study was to try to combine clinical roentgenographic and histopathologic criteria of fibrous dysplasia of jaw bones on the basis of a large series of cases. Possibilities for a unified terminology diagnosis and therapy could be thus increased.

CASE MATERIAL

The case material consisted of 20 patients with fibrous dysplasia of jaw bones, all of whom underwent a detailed clinical roentgenographic and histopathologic examination Their age ranged from 7 to 80 years. Of the 20 patients, 12 were women and 8 were men In 16 cases the disease was localized to the upper jaw whereas only four presented lower jaw lesions (Table 2) In order to reach the unity of judgement we sought a retrospective study was made of the whole roentgenographic and histopathologic material

Clinical features

The clinical features were uncharacteristic Minor lesions produced no subjective complaints, and were sometimes detected by a dentist in the course of a routine roentgenographic examination The first and most frequent symptom was a more or less pronounced swelling of the jaw bone In the mandible the swelling was commonly localized to the corpus and more seldom, to the ramus or the temporomandibular joint In the upper jaw the alveolar process was involved to a varying extent Lesions were often localized about the canine fossa although they sometimes involved the region around the zygomatic bones, where they generally produced facial

TABLE 2 *Histologically verified cases with fibrous dysplasia of the jaw bones*

Case	Age at diagnosis (years)	Sex	Localization	No. of operations	Retrospective roentgenographic diagnosis
1	49	♀	Maxilla	1	+
2	9	♂	Maxilla	1	
3	18	♀	Mandible	1	
4	32	♂	Maxilla	1	
5	45	♀	Maxilla	3	+
6	7	♀	Maxilla	2	+
	57	♀	M xilla	1	+
8	14	♀	Maxilla	2	
9	16	♀	Mandible	2	+
10	11	♂	Mandible	1	
11	80		Maxilla	1	
12	27	♂	Maxilla	2	+
13	41	♂	Maxilla	1	−
14	21	♂	M xilla	5	+
15	29	♀	Maxilla	1	
16	16	♂	Maxilla	2	
17	30	♀	Maxilla	1	+
18	45	♀	Maxilla	1	−
19	71	♀	Maxilla	1	
20	11	♂	Mandible	1	

asymmetry Frequently lesions localized to the upper jaw extended diffusely in the walls of the maxillary sinus, causing it to be constricted. The swelling was not mobile and untender to palpation, and usually without marked contour The mucous membrane of the mouth was normal within the affected regi n. The teeth were occasionally displaced, and produced mal occlusion. Although pain sometimes occurred, it was seldom constant or severe The swelling grew ery slowly and in some cases it had been noticed by the patient years before consulting the doctor or the dentist.

The clinical features are uncharacteristic and roentgenograms can vary In half of the cases, fibrous dysplasia could be diagnosed at the retrospective roentgenographic study (Table 2) Nevertheless, histopathologic examination hould be required for the diagnosis to be considered as conclusive For this reason, surgical biopsy was performed in every suspected case of fibrous dysplasia.

If only a minor local focus was detected, it was radically removed. If on the contrary the disease showed major diffuse expansion, a partial resection was performed, removing those parts f the jaw bone which caused func tl nal or cosmetic disturbances. No radical resection was undertaken. If the lesion recurred a new conservative operation was performed.

Altogether 13 of the 20 patient were operated on once only and the other seven patients twice or more (Table 2) The latter cases, cannot, howeve



F g 1 ( ) Bo ged 11 ears (case 10) Earl cha ge mandibl with filiform rarefica-tio without conne ion with root f the teeth. Slight increase i density al ng lower border of mandibl with bl rri g or cort spongiosa j ncti n. (*b*) Same patie t 11 years l t Thickeni g f th bod f the m ndibl which ha acq ired the density of *cortical bone The teeth remai od the second molar ha erupted d ri g th observ tion* period.



Fig 2. Girl, ged 16 years (case 9) Earl chang l mandibular ramus, with destruction od periosteal bo deposition. N rmal left ram ( ) for comparison with (*b*) the affected right ram (tomogram)

be considered as actually recurrent. This is because for reasons mentioned above the first intervention was always limited to a cosmetic" operation

Although some authors (Coley & Stewart 1945 Sutro, 1951 Gold, 1955) have stated that there is a risk of the process undergoing malignant transformation, this has been denied by most of the authors referred to in the present paper None of the 20 cases examined by us showed any malignancy and none presented polyostotic fibrous dysplasia Thus the disease involved no skeletal area but the upper and lower jaw bones. Furthermore no symptoms of Albright disease (polyostotic fibrous dysplasia, abnormal skin pigmentation and precocious puberty) were observed in any of the cases.

Roentgenographic features

The roentgenographic features of fibrous dysplasia of the lower jaw are less known than those of the lesion involving the upper jaw which has more often been described in the literature (Jaffe, 1961) Changes localized to the lower jaw were confirmed by us in four cases, two of which had been observed over a long period These cases had very similar roentgenograms.

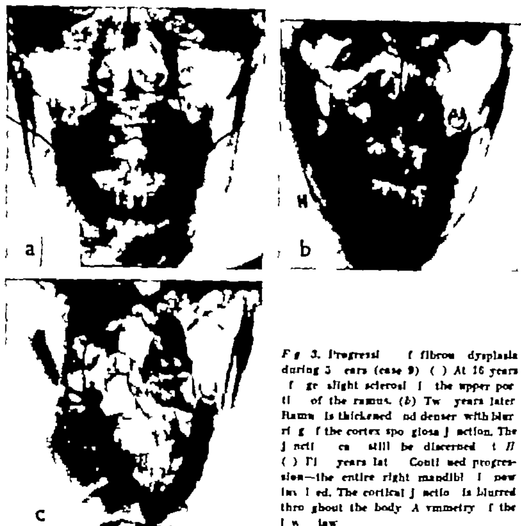


Fig. 3. Progressi f fibrous dysplasia during 5 ears (case 9) ( ) At 16 years f ge slight sclerosi f the upper por ti of the ramus. (*b*) Tw years later Ramu is thickened nd denser with blur ri g f the cortex spo giosa j action. The j ncti ca still be discerned t *H* ( ) Fi years lat Conti ued progression—the entire right mandibl I new inv l ed. The cortical j nctio is blurred thro ghout the body A ymmetry f the l w jaw

It seems, therefore possible to establish a fairly accurate diagnosis based on roentgenographic material

At early stages f the process, predominantly destructive changes were visible in the f rm of either elongated and filiform or round thinnings in the spongi sa, with partly distinct and partly undefined delimitati n from the surrounding bone These destructions were not related to the roots of the teeth but *were generally separated from them by a zone of normal bone* Even at this early stage the borderline between spongiosa and corticalis was obliterated (Fig 1 *a*) A picture of a probably very early lesion in the mandibular ramu showed on the one hand a destruction of rather indistinct contour and on the other hand layers of periosteal bone which aroused suspicions of osteomyeliti (Fig 2 *b*) At later stages, the mandible became wider and sclerotic having throughout the same density as cortical b ne (Figs. 1 *b* 3 *b* and 3 *c*) In one patient who was under observation from 9 to 20 years of age the molars developed normally despite the bone in this



Fig 4 F brous dysplasia I upper jaw with thickening f th lveola process nd reduc tion f the sl (case 14)

region being considerably affected (Fig 1) In this case as well as in the aforementioned one the process started posteriorly in the mandibular ramus and developed anteriorly over the median line (Fig 3) and in one of them the entire mandible was virtually altered at the age of 29

Typical roentgenographic finding in the upper jaw are shown in Figs. 4 and 5 The alveolar proces of the maxillas was wider than on the normal side and the maxillary sinus was narrowed from below by the dense bone of the process. An intraoral film disclosed that the distance between the tooth roots and the floor of the maxillary sinus had increased, and that the bone in the alveolar process was more compact than normally

Pathologic features

The macroscopic picture was characterized by a greyish white tissue, inv lving mainly the medullary cavity of the jaw bones. This caused deform ity of the bone and sometimes a distinct thickening was apparent. The affected tissue s consistency ranged from soft to very hard. Even within different parts of the same lesion, the consistency sometimes varied con siderably The affected regions were clearly delimited from surrounding normal bone tissue but no encapsulation was present.

The microscopic picture revealed two main tissue components, i.e fibrous tissue and thin newly formed osseous trabeculae Degenerative changes with

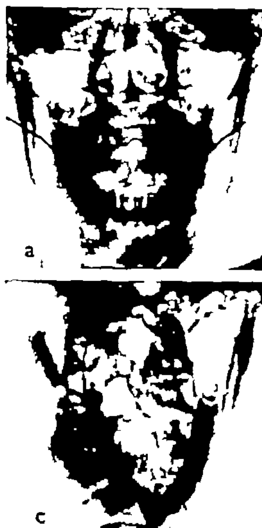



Fig 3. Progres ion of fibrou dyspl sia during 5 ears (case 9) ( ) At 16 years of ge slight sclerosi i the upper por ti n f the ramus. (*b*) Two years later Ram i thickened nd denser with blur ri g f the cortex-spongiosa j ction. The j nction ca st ll be discerned at *H* ( ) Fi years later Conti ued progres ion—the entire right mandibl i now invol ed. The cortical j nction is blurred throughout the body A ymmetry of the lower j w

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Fig 6 Early fibrous dysplasia i upper jaw f 14-yea -old girl. Cellula co nective tissue with scattered island f bone formati (interpreted as metapl si) Moderat umbers f giant cells. Case 8, Gieson, 270.

relation was present between the patient s age and the histologic appearance of the lesion. In young patients, whose lesions were probably still at an early stage the connective tissue was highly cellular and contained giant cells. In these cases, the newly formed, immature osseous trabeculae were very few and had no osteoblastic activity (Fig. 6)

In patients from 30 to 40 years of age the lesion seemed to have reached an intermediate stage in its development. The bone tissue had formed much thicker trabeculae, which presented signs of lamination in places. Osteocytes nd single osteoblasts were sometimes present. At this stage of development, the connective tissue was less cellular than in lesions of shorter duration (Fig 7)

Patients over 40 years presented changes that we interpreted as late stages of fibrous dysplasia. In these lesions, the osseous tissue consisted of thick, clearly laminated trabeculae with osteoblasts. The connective tissue contains relatively few cells, which decreased in proportion to the osseous elements (Fig 8)

In several patients, we had the opportunity of following the development of fibrous dysplasia by means of repeated roentgenographic and histopathologic examinations. In one case we could, for 18 years, follow the course of fibrous dy plasia in a man who was 21 when first seen The aforementioned

relation between the histopathologic appearance of the condition and the age of the patient—and probably also of the lesion—could then be observed in one and the same case (Fig. 9).

DISCUSSION

In our cases of fibrous dysplasia of jaw bones, the majority were localized to the upper jaw. This is in agreement with most earlier reports (Table 1). Billing & Ringertz (1946) suggested, as an explanation of the more common localization of fibrous dysplasia to the upper jaw, the fast growth of the tuber maxillae region in puberty, when fibrous dysplasia of the upper jaw usually starts.

The sex distribution in our series (12 women and 8 men) is essentially the same as in previous studies (Table 1) (Billing & Ringertz, 1946; Jaffe 1961; Houston, 1965).

We found that fibrous dysplasia of jaw bones could only exceptionally be diagnosed clinically. Objective as well as subjective symptoms, are extremely vague and uncharacteristic; presumably one of the reasons why this form of fibrous dysplasia is often diagnosed fairly late.

The roentgenographic features of fibrous dysplasia of the lower jaw have hitherto been little known. Nevertheless, reports of fibrous dysplasia have generally been accompanied by roentgenograms (Billing & Ringertz, 1946; Jaffe 1953; Houston 1965). Like preceding studies, ours shows that roentgenographic diagnosis of these lesions may be of great value. Since roentgenograms have been considered to be uncharacteristic at early stages of fibrous dysplasia of jaw bones, the examination has so far had little value in such cases. At later stages, on the contrary, roentgenograms are much more useful. We were able to observe the development of some cases of fibrous dysplasia of lower jaw bones through roentgenographic examination over a fairly long period. This implies an increased possibility of establishing the diagnosis on this basis even at an early stage. Nevertheless, a definite diagnosis can be made only with the help of biopsy. Thus, in our series, a definite diagnosis could be established roentgenographically in only half of the cases, despite the radiologist being experienced, and well acquainted with the disease (Table 2).

The great differences between the histologic features of fibrous dysplasia at early and late stages of development explain why they have been regarded as entirely different skeletal diseases and, consequently, received different names. In view of the small number of well-documented cases observed clinically and roentgenographically over a long period, it has been impossible to reach uniformity in the diagnosis.

Jaffe (1953 and 1961), among others, grouped these skeletal diseases under the name "fibrous dysplasia of jaw bones". He also emphasized the possibility that completely ossified formations of the osteoma type could be included in this group. This assumption is strongly supported by our ex-

perience The cases we have observed over a long period (up to 18 years) show that, after an initial phase of development characterized by intense proliferation of connective tissue fibrous dysplasia enters a stage in which the osseous tissue attains maturity but without further growth. The lesion then assumes the histologic appearance of an osteoma. Houston (1965) found no signs of such a development, although he did not deny its possi bility In our opinion, it is essential to create a uniform terminology for these changes in jaw bones. Earlier investigations, e.g. Schlumberger s and Jaffe s, as well as our study indicate that "fibrous dysplasia of jaw bones" should be the term applied to the condition Its general acceptance and abandon ment of all synonyms still used should lay the foundations for a more uni- form diagnosis and therapy

The present study has not contributed to clarifying the etiology of the disease. Nothing indicated the existence of hereditary traumatic or infec tious factors. The age distribution of the patients, as well as the process of development, nevertheless seem to support the view that fibrous dysplasia of jaw bones is to be regarded as a developmental defect in osseous tissues (Billing & Ringertz, 1946 Jaffe 1953, 1961 Berger & Jaffe 1953)

Neither the present study nor previous ones have disclosed sarcomatous changes in fibrous dysplasia of jaw bones. Consequently we believe that treatment should be confined to partial resection of lesions causing func tional or cosmetic disturbances.

ZUSAMMENFASSUNG

Es wird eine kli ische, röntgenologische und histopathologische Untersuchung von 20 Fällen mit fibröser Dysplasie im Kiefer vorgelegt, und die Resultate werden im Anschluss an ei e Durchsicht der Literatur auf diesem Gebiet disku- tiert. Im vorliegenden Material wa die Verteilung der Lokalisation im Oberkiefer U terkiefer = 16 4 und die Verteilung auf die Geschlechter M F = 8 12. Die Pathogenese wird anhand röntgenologischer und histopathologischer Bilder von Fällen beleuchtet, von denen einige mehrere Jahre hindurch kontrolliert wurden. Röntgenologisch ist di Diagnose m schwierigsten im frühen Entwicklungs- stadium zu stellen, und muss immer mit ei er histopathologischen Untersuchung bekräftigt werden. Die fibröse Dysplasi im Kiefer ist völlig gutartig und wird nach und nach stationär daher ist die richtige Behandlung eine konserv tiv hir urgische.

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POSTURE AND PRESSURE WITHIN THE INTERNAL JUGULAR VEIN

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The blood pressure of the bulb of the internal jugular vein was studied in healthy v lunteers in different postures. The positions examined were horizontal, dorsal and lateral, and also several elevation degrees of the upper part of th bod The influence of compression of th neck veins upon the venous pressure was also studied. A compression of 25 mm Hg produced the same increase in enous pressure as that caused by a change from the itting to the recumbent position. The investigations are intended as the basis for further studies of the variati ns of con gestion occurring in different body positions in the mucosa of the nose, the Eustachian tube and the middle ear

The vessels of the mucosa of the nose, the Eustachian tube and the middle ear open into the internal jugular veins. In different body positions the hydrostatic variations of the pressure within the internal jugular veins affect the degree of filling of the vessels. Such mucosal phenomena have been reported e.g. by Perlman (1939) who studied the closure of the patu lous Eustachian tube in the recumbent position. Analogous observations have been made on the nasal mucosa (Rundcrantz, 1964) and on the mucosa of the middle ear and Eustachian tube (Ingelstedt *et al* 1967)

The aim of the present investigation was to study the variations of the pressure in the internal jugular vein in different body positions from the recumbent to the erect We also intended to find a method to increase rapidly the pressure within the jugular vein in the vertical position to that prevailing in the horizontal position The present study was at the same time the basis for further investigations of the effect of varying venous pressure on the mucosa of the nose, th Eusta hian tube and the middle ear

SUBJECTS AND METHOD

Five healthy men, aged 24–47 were examined. They had no history of disease of the heart or of the vascular system. Their ECGs and arterial blood pressures were normal The pressure within the bulb of the internal jugular vein was mea ured by a differential pressure transducer (EMT 34

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Elema-Schönander Solna, Sweden) via a Cournand catheter No 7 The catheter was introduced via a cubital vein according to a technique described by Lindell *et al* (1962) The catheter was advanced under fluoroscopic control in two projections until the tip was lodged exactly at the level of the external ear canal The pressure transducer was calibrated with a water manometer and was carefully placed at the level of the catheter tip The pressures obtained were recorded on a multi-channel recorder (Mingograph 81 Elema-Schonander) At all determinations the level of the catheter tip was measured in relation to the right atrium of the heart The atrium was in all positions supposed to be situated on a transversal axis 5 cm dorsally to the insertion on the sternum of the fourth rib. This axis may be slightly different from the phlebostatic axis proposed by Winsor & Burch (1945) but it was used for convenience.

In order to increase the pressure within the jugular vein an inflatable cuff was used similar to a torniquet for arterial blood pressure determinations. The cuff had a width of 6 cm and a length of 50 cm and was applied round the neck. It was inflated by a variable electric fan, the pressure of which was controlled by a mercury manometer The arrangement allowed the pressure inside the cuff to rise instantaneously from zero to a desired level

PROCEDURE

After catheterization 4 of the subjects were moved to a folding chair where they could be placed with the upper part of the body in elevations between half-sitting (65) and horizontal (0) The venous blood pressure was then measured at the elevations 0 10 20 30 40 55 and 65 In the latter position the cuff was loosely applied. In each of the mentioned elevations the cuff was then inflated to 11 17 23 and 30 mm Hg and the resulting venous pressure increase was recorded. In the fifth volunteer the pressure in the left internal jugular vein was determined in different horizontal positions, i.e dorsally and laterally on the left and on the right side—with and without a pillow under the subject s head. Subjects 1 to 4 experienced only slight discomfort due to the catheter while the fifth subject reported distinct aural pain when he turned into the lateral positions. This is the reason why only one examination was performed in this way

RESULTS

Fig 1 shows the data obtained in all the subjects in different positions. A change of the elevation from 65 to 20 caused a pressure increase of only 2.1 mm Hg, a further change to 0 caused an additional increase of 4.5 mm Hg on the average At elevations of 20 or less the change of venous pressure corresponded closely to the change of hydrostatic level In all positions, inflation of the cuff caused an immediate rise of the pressure in the

TABLE 1 *The increase of pressure (mm Hg) in the internal jugular vein and inflation of the cuff (mm Hg) in different postures (mean of 4 subjects)*

Cuff pressure	Rise of venous pressure			
	65	30	20°	0
11	2.4	2.1	2.4	2.0
17	3.9	3.4	4.3	3.8
23	6.0	5.5	6.5	5.8
30	8.2	7.6	8.	9.0

internal jugular vein amounting to approximately 25% of the pressure produced by the fan (Table 1)

The results of the tests on the fifth subject are shown in Table 2 and Fig. 1 The lower the tip of the catheter in relation to the right atrium, the higher was the pressure of the vein The highest pressure was recorded when the subject was lying on the left side without a pillow The position of the catheter was then in the declive part of the head.

DISCUSSION

The pressure measurements within the internal jugular vein were performed in different postures and at compression of the neck veins effected by an inflatable cuff The study was intended to be the basis for further investigations of the flow-resisting properties of the nose and of the Eustachian tube at varying degree of congestion in different postures. It was shown that in the horizontal position, when the bulb of the internal jugular vein was a few centimeters below the level of the right atrium of the heart, the venous blood pressure was between 4.5 and 11 mm Hg These values are in good accordance with the results reported by other investigators (Doupe *et al.*, 1938 Warren & Stead, 1943 Woringer *et al* 1954 Ingelstedt *et al* 1967) When the body position changed and the jugular vein was above the level of the right atrium, the pressure in the vein decreased.

TABLE 2

Posture	Level of catheter tip (cm) in relation to the right atrium	Venous pressure (mm Hg)	Venous pressure ($cm\ H_2O$)
Left side horizontally	−8	10.1	+13.7
Dorsal	−6	+8.6	+11.7
Left side with pillow	−4	+8.0	+10.9
Dorsal with pillow	+2	+2.4	+3.3
Right side horizontally	+3	+1.5	+2.0
Right side with pillow	+9	−0.6	−0.8

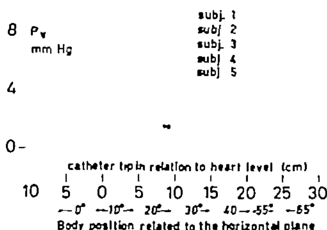


Fig. 1 Th e ve pressure in relation t body positi n nd the lev l of the catheter The sc le for body position is ot valid in ubject No. 5

Owing to the tendency of the veins to collapse the pressure decrease in posi tions above heart level is considerably lower than the corresponding hydrostatic component (Alexander 1963) In our experiments the catheter tip was lodged within the bulb of the jugular vein, which cannot collapse due to the bony external support. This fact is essential for accurate pressure deter minations. In subjects 2 and 5 pressures below zero were encountered, but as a rule there seemed to be a slightly positive pressure in the erect position The studies on subject 5 in different recumbent positions revealed that the pressure variations corresponded very closely to the hydrostatic pressure components, represented by the different levels of the catheter tip as measured from the right atrium

There are two evident reasons why the venous pressure did not increase by more than 25% of the cuff pressure First the cuff was too narrow for the pressure transmission through the neck tissues to be complete Secondly there are vertebral and spinal venous plexa which are protected from external compression. Attempts to perform cerebral phlebographic examina tions by injection of X ray contrast medium cranially to a slight compression have repeatedly shown how the contrast very rapidly disappears, most of it via intraspinal venous plexa (Olin, 1968)

The pressure prevailing in the dorsal recumbent position could be reproduced in the sitting position by an inflation of the cuff of approximately 25 mm Hg

It may be concluded from the investigations that if a low venous pressure in the head and neck should prove desirable in a patient, he should be advised to take up a position during bed rest with an elevation not less than 20 A higher elevation causes only little additional pressure decrease It may also be of some importance in the lateral position that the declive half of the body has a higher venous pressure than the opposite part.

ZUSAMMENFASSUNG

An gesunden Versuchspersonen wurde der Blutdruck im Bulbus der V. jugulari interna gemessen um zu untersuchen wie dieser Blutdruck von Änderungen der Körperstellung abhängt. Die untersuchten Körperstellungen waren die waagerechte Rücken und Seitenlage sowie mehrere Lagen über der Horizontalen Ferner wurde der Einfluss einer Kompression der Halsvenen auf den venösen Blutdruck studiert Der ohne Kompression in waagerechter Körperstellung gefundene Druck konnte durch Kompres ion in senkrechter Stellung reproduziert werden Die Arbeit wurde im Hinblick auf fernere Studien der verschiedenen Grade von Schleimhautschwellungen durch eführt, die sich in der Nase, in der Tuba Eustachii und im Mittelohr entwickeln können

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VELOPHARYNGEAL INCOMPETENCE POST TONSILLO-ADENOIDECTOMY

An Electromyographic Study

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Out of 81 patients suffering from velopharyngeal incompetence nine persons were found to have developed nasal speech (Rhinolalia) after having undergone tonsillo-adenoidectomy The velopharyngeal incompetence was corroborated by cinefluorography and respiratory studies. These nine patients underwent an electromyographic study as well, which revealed sub-mucous cleft palate in six of them. The role of tonsillo-adenoidectomy in breaking down compensated palato-pharyngeal incompetence is discussed.

Velopharyngeal competence is indispensible in normal speech performance The soft palate and the posterior wall of the pharynx contribute to the formation of the sphincter which separates the oropharynx from the nasopharynx thus preventing unfavorable nasal speech (Morley 1966) Velopharyngeal incompetence may be the result of an immobile or short soft palate or a large palatopharyngeal orifice The sequela to one of these defect is hypernasality (Rhinolalia) The main cause of this incompetence is a cleft palate other causes are deformities of cranial base paralysis of the palate and submucous cleft palate Tonsillectomy and adenoidectomy are considered as possible causes of velopharyngeal incompetence by their impact on the size of an otherwise compensated palatopharyngeal orifice (Chase 1960)

Our purpose in this study was to investigate the condition of the soft palate musculature in patients with hypernasality following tonsillo-adenoidectomy

MATERIALS METHODS AND RESULTS

During the period December 1 1967 and June 1 1968, 81 hypernasal patients (age 4–10 years) were seen in our cleft palate clinic. In nine of these their parents became aware of hypernasality after tonsillo-adenoidectomy was performed Before this operation all the children had normal speech Clinical examination revealed obvious hypernasality which was



Fig. 1 Electromyography of the midline poi t of the soft palat in six patient shows lectrical silence which implies absence f f ncti ning motor its.

Fig. 2. Numerou ction potential depicted from the middle third of the soft palat on both sides i ine patients. N rmal lectromyographic pattern. Calibration Amplitude, 100 microvolt /cm; duration, 5 millisec/cm.

verified by evaluation of the speech pathologist. Examination of the soft palate did not reveal any structural or functional abnormalities

Velopharyngeal incompetence was corroborated in all the patients by two tests (*a*) cinefluorography which showed imperfect palate to pharynx touch-closure during spontaneous speech (Yules & Chase, 1968) (*b*) respiratory studies which demonstrated significant nasal air leak.

All the patients underwent electromyographic studies in order to evaluate the muscles of the soft palate A Meditron EMG unit was used and bipolar electrodes were employed. The electrodes were inserted into the following points successively on each side of the middle third of the soft palate (the level of the dimple of the levator palati) and in the midline of the soft palate (Lubker 1968)

The patients were instructed to say ah" and the potentials exhibited on an oscilloscope screen were filmed.

In six patients no potentials were recorded from the midline points of the soft palate This finding implies absence of functioning motor units (Fig. 1) In these patients the records from the middle third of the soft palate were normal (Fig. 2) All the examined points of the other three patients were within normal limits.

DISCUSSION

Of importance is the finding of absence of motor units in the midline of the soft palate in our six patients because this p ttern is typical of the submucous cleft palate (Rees *et al* 1967) Submucous cleft palate is defined as an imperfect muscle uni n in an otherwise intact soft palate The em-

bryonic defect is a failure of union of mesodermal elements in the palatal plates across the midline of the developing soft palate

These patients although they had an anatomical basis for velopharyngeal incompetence and resultant hypernasality compensated very well before the tonsillo-adenoidectomy operation they could bring the soft palate to touch adequately the posterior wall of the pharynx—perhaps by compensational overactivity of the constrictor pharyngeus. The tonsillo-adenoidectomy operation broke down this vulnerable and unstable situation and revealed the underlying pathology

This data leads us to propose that every patient who is a candidate for tonsillo-adenoidectomy should have the condition of the soft palate investigated in order to detect structural or functional abnormalities. In addition to clinical examination an electromyographic test of the soft palate musculature is recommended

ZUSAMMENFASSUNG

Von einundachtzig kranken die an weicher Gaumen Pharyngeal-Unfähigkeit leideten, wurde in neun Menschen gefunden dass sie nach Tonsillektomien Adenotomien, die Entwicklung von Nasensprachen (Rhinolalie) entstand. Die Unfähigkeit des weichen Gaumens und des Schlundes hat sich bei Kinematografie und respiratorischen Untersuchungen bestätigt. In sechs von diesen neun kranken machte sich ausserdem durch eine elektromyografische Untersuchung eine sub-muköse Gaumenspalte bemerkbar Die Rolle der Tonsillektomien-Adenotomien in der Schwächung der kompensierten Defekten des weichen Gaumens und des Schlundes wird hierbei diskutiert.

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POSTURE AND EUSTACHIAN TUBE FUNCTION

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It has been well known for a long time that the patency of the Eustachian tube is affected by body position, particularly in patients with patulous tubes. The ventilatory capacity of the Eustachian tube was studied quantitatively in subjects with normal tubal function in different postures and during compression of the neck veins. In the horizontal position or during compression of the neck veins with 35 mm H_2O the volumes passing through the tube during deglutition were reduced to /.

In a body elevation of 20 or 30 the tubal function quantitatively decreased to / When the subject was lying on one side the tube of the downturned ear seemed most affected. In subjects who caught colds the consequence of horizontal position was very marked.

Clinicians are familiar with the fact that the symptoms of acute otitis media often start at night when the patient has been in bed for some hours. During upper respiratory infections or after intratympanic surgery when tubal dysfunction may be expected, patient should be advised to take up body position of not less than 20 above the horizontal plane in order to prevent middle ear complications due to the positional influence on the tubal function. In selected cases tests of the tubal function might be performed also in the recumbent position.

In 1867 Lucae in Germany reported that variations of the patency of the Eustachian tube took place at different positions of the head. He found that a greater overpressure had to be produced against the closed nose if his head was bent backward than if it was bent forward in order to force air through the tube. Hartmann (1879) recorded the overpressure in the rhinopharynx required to open the tube during Valsalva's manoeuvre. He also noted that a higher pressure was needed when the head was bent than when it was held upright. Perlman (1939) reached the same conclusions in his studies of the influence of body position on patulous and normal Eustachian tubes. Perlman assumed that the closure of the patulous tube and the reduced patency of the normal tube which occurred at a change from the erect to the recumbent position were due to lymphatic or venous stasis in the vessels of the tubal mucosa. Several investigators have later made similar observations on the patulous tube. It has become a diagnostic sign that the symptoms of this disorder are relieved in the horizontal posi-

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bryonic defect is a failure of union of mesodermal elements in the palatal plates across the midline of the developing soft palate.

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Fig 1 The middl ear model. ec= xternal ea canal with the rubber disc nd catheter inserted.

is directed away from the middle ear when the pressure is negative the direction of the air flow is the opposite The same air flow passes through the measuring device and is recorded, negative (−) and positive (+) respectively Δ Vmuc produces an air flow through the catheter when the tube is closed. An increase of the volume of the middle ear mucosa will cause an air flow from the middle ear through the measuring device (+) a decrease leads to the opposite (−)

The air flow and volume measuring device

Fig. 2 is a block diagram of the air flow and volume measuring device, which is a modification of that described by Ingelstedt *et al.* (1967)

The catheter air tightly inserted into the bony part of the external ear canal, is connected to the flow meter This is a pneumotachograph consist ing of a single capillary resistor The pressure drop across the resistor is measured by a differential gas pressure transducer (EMT 32, Elema Schönander Stockholm, Sweden) The flow meter and the transducer are sur rounded by a thermostated and airtight metal container for minimizing the

Fig 2 Block diagram f the flow nd lume measuring device. *A B C* and *D* indi t the different part f the t bing tem.

tion (Moore & Miller 1951 Pulec & Simonton, 1964 Flisberg, 1966) Investigating healthy military aircraft pilots in a pressure chamber Ingelstedt *et al* (1967) confirmed the reduction of the ventilatory capacity of the tubes even in these subjects.

It is thus quite evident that the pressure-equalizing ability of the Eustachian tube is affected by different body positions in healthy persons. It would therefore be logical to assume that the tubal function is still more affected in patients suffering from diseases of the middle ear or of the upper respiratory airways. This would be analogous to the increased blockage of the nasal cavities in rhinitic patients and the mucosal congestion in children with subglottic laryngitis, which occur in the recumbent position (Rundcrantz, 1964 and 1969 Ingelstedt *et al.*, 1967)

Ingelstedt *et al* (1967) seem so far to be alone in considering that the effect of the horizontal body positions on the tubal function would possibly constitute a factor contributing to the development of acute or chronic diseases of the middle ear

Aim of the Investigation

The aim of the present investigation was to study to what extent different body positions would affect the patency of the Eustachian tube and to try to analyse the mechanisms behind the phenomenon

Equipment and Method

The following symbols are used

Patm, atmospheric pressure
Pfan, pressure produced by a fan acting on the recording system.
Pm, middle ear pressure
Pneck, pressure inside the neck vein cuff
ΔVmuc the volume changes of the mucosa lining the middle ear space
V̇r the instantaneous flow rate through the flow meter
V̇t, the instantaneous flow rate through the Eustachian tube
Vr the air volume passing through the resistor of the flow meter
Vt, the air volume passing through the Eustachian tube
ΔVsyst, the gas volume changes of the system of measuring device and middle ear space when Pfan is acting on it

The middle ear

Fig 1 shows a middle ear model with a perforated drum A polyethylene catheter with a circular rubber disc of 2 mm thickness mounted around its end is inserted into the inner bony part of the external ear canal, connecting the middle ear space with the measuring device Through the catheter Pm can be changed in relation to Patm If the tube opens during deglutition a flow of air will pass through the tube When Pm is positive the air flow

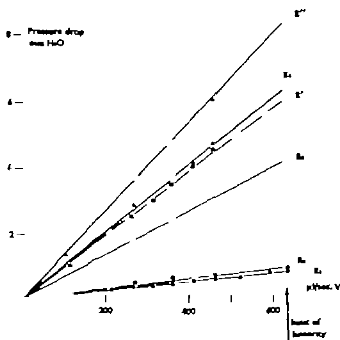


Fig 3 The pressure drop across the measuri g device nd the different part of the tubing system related to the air flow (see text)

Properties of the Complete Flow and Measuring Device

Linear response to low and high velocities 0–625 microlitres/second. The tubing system connecting the resistor with the pressure fan and with the middle ear space is seen in Fig 2

R the resistance of the resistor (B–C)

R the resistance of the catheters connecting the resistor with the fan and with the ear canal (A–B and C–D)

Two different lengths of the ear canal catheter (D–F) PE 200 were used.

R the resistance of short catheter (58 cm)

R the resistance of long catheter (82 cm)

$R + R_3 + R = R'$ $R + R + R = R$

The values of different R within the limits of linearity are seen in Fig. 2 Sinusoidal frequency response was flat over 20 cps, Fig. 4 Transient response 90% response in 8 msec. Calibration accuracy 1 μl$\pm$5%

MATERIAL AND PROCEDURE

Sixteen subjects were investigated, 8 men and 8 women, aged 11–
All of them had central dry perforation of the tympanic membrane In 12 cases there were post-otitic sequelae In 4 cases the perforation

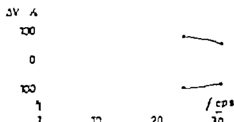


Fig 4 The sinusoidal frequence response of the complet flow and measuri g device.

traumatic Two of the latter were performed by incision of the drum and a short polyethylene tube (PE 200) of collar button" shape was inserted through the incision The size of the perforations in all other subjects was bigger than the diameter of PE 200

The diameter of the catheter disc had to be chosen individually in order completely to block the lumen of the ear canal, which was filled with high vacuum grease outside the disc to secure air tightness. The whole procedure was performed without anaesthesia but caused the subjects very little discomfort

Calibrations of the measuring device were made before and after each examination The air tightness of the whole system was controlled continuously and leakage was discovered immediately and could be corrected.

The subjects were placed in a folding-chair adjustable at different elevations from the sitting to the horizontal position. First of all the tubal functions of the subjects were tested in the sitting position. A prerequisite for the following examinations was that all subjects could equilibrate a negative and a positive intratympanic pressure of 4 cm H_2O during deglutition.

The air flow through the tube ($\dot{V}t$) was, however not allowed to exceed at any moment the limit of the linear response of the measuring device This occurred in 5 tested subjects, 4 of whom had patulous tubes. These subjects were excluded from the experiments and the material During the following examinations a positive intratympanic pressure was always applied, 2 cm H_2O in 13 cases, 4 cm H_2O in 3 cases. The reasons for this are discussed below

The subjects were examined under the following conditions

1 Sitting erect (90)
2. Lying dorsally elevation 30 (12 subjects)
3. Lying dorsally elevation 20
4 Lying dorsally horizontally (0)
5 Sitting erect, neck veins compressed by cuff 15 cm H_2O (7 subjects)
6. Sitting erect, neck veins compressed by cuff 35 cm H O
7 Lying laterally left and right, elevation 20 (8 subjects)

In every situation 10 recordings were made After lying down or after neck compression, condition no. 1 was re-established for 5–10 minutes. In

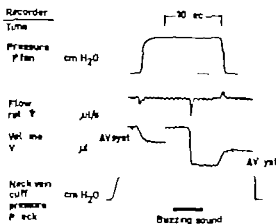


Fig 5 A example f imultaneous recordi g of time, Pfan, V V and Pneck.

order to avoid exhaustion of the muscles opening the tube an interval of 20 minutes was made after a period of 40 minutes examination. The examinations had to be finished in about 2 hours, after which the subjects often reported aural discomfort Every subject, except the 2 patients with polyethylene tubes through their drums, was examined at least on two occasions, but owing to the time-consuming nature of the procedure it was not possible to repeat the whole programme Most observations have therefore been made with the subjects lying dorsally 20 and 0 and with neck compression 35 cm H_2O In the course of the investigation five of the subjects fell ill with upper respiratory infections and were re-examined during the infection and a third time one month later

RESULTS

Fig. 5 is an example of a recording of time Pfan, $\dot{V}r$ Vr and Pneck. ΔVsyst is an expression of the changed gas volume of the measuring device and of the middle ear space when Pfan acts upon them and is of no interest in the present investigation.

In Fig 6 the recording of Vt in one of the subjects during one examination have been drawn in a diagram In the 30 position there was no visible change but in the 20 and 0 positions as well as during neck compression with 35 cm H_2O a reduction of Vt took place ΔVmuc was also recorded on every change in position and was greatest between 90 and 0 and during compression with 35 cm H_2O It was often observed that after a change of position it took a few minutes before a steady tat was established Therefore when calculating the mean values of Vt the first two recordings in every new situation were excluded. When, f r example Vt in the 90 p si tion (Vt 90) was compared with Vt in the horizontal position (Vt 0) the mean of Vt 90 wa calculated fr m th recordings made before nd after

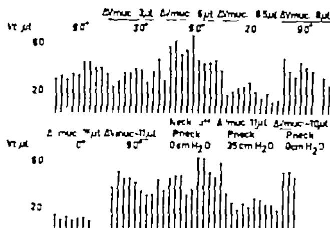


Fig 6 Vt i different bod positio nd d ring neck l compression i one subject. J Vma w lso recorded on every cha ge f position.

the subject was lying horizontally In this way the changes of Vt in different positions and during compression of the neck veins were estimated as the percentage of Vt 90 Table 1

Mean of Vt 90 varied between 10? and 7µl/deglutition in different subjects.

In the positions of 30 and 20 or when compressing the neck veins with 15 cm H_2O Vt decreased by $^1/_3$ as a mean In the recumbent position or during neck vein compression with 35 cm H_2O Vt decreased by $^2/_3$.

There was no significant difference between the effects on Vt in the positions of 30 and 20 and during compression with 15 cm H O The reduction of Vt in the horizontal position and with the neck vein compression of 35 cm H O differed significantly from the above mentioned values (0 005 >

TABLE 1

Position	Vt, of Vt 90	Significance of difference from Vt 90
30	72.6, s.e. = 6.3, s.d. = 23.6, = 14	$0.005 > p > 0.001$
20	63.7 s.e. = 4.0, s.d. = 21.2, n = 28	$p < 0.001$
0	38.1 s.e. = 4.9, s. = 22.1, n = 20	$p < 0.001$
Compression of the neck		
15 cm H_2O	71.9, s.e. = 10.1, s.d. = 33.5, n = 11	$0.025 > p > 0.010$
35 cm H_2O	41.3, s.e. = 5.2, s. = 23.6, = 21	$p < 0.001$

TABLE 2

Position 20°	Vt, % of Vt 90°
Ear up	56.1 ± s.d. 27.7 s.e. 7.4 n = 14
Ear down	45.8 ± s.d. 28.4 s.e. 7.6 n = 14

$p > 0.001$) There was no difference between the effect of the 0° position and of the compression with 35 cm H_2O. Great individual variations were seen, as appears from the figures of s.d.

Eight subjects were examined in the position of 20° above the horizontal line lying on their sides with the ear to be studied alternately turned upwards or downwards, Table 2. The figures show that the effect on Vt tended to be greater in the latter than in the former case ($0.1 > p > 0.05$).

Among the subjects examined during upper respiratory infections, 2 could not open the Eustachian tube at all for a Pfan of 4 cm H_2O in the sitting position. The other 3 could equilibrate in the sitting position when Pfan was 2 cm H_2O. Table 3 shows the effect of different positions on these subjects in healthy condition and during infection.

During infection a general reduction of Vt 90° was seen in 3 subjects but the effects on Vt in the 20° and 0° positions were very marked. None of them could open the tube in the horizontal position. After 10 minutes in this position the tubal function did not recover until after 10–35 minutes in the erect position.

TABLE 3

Subject No.	Vt 90° µl	Vt 20° µl	% of Vt 90°	Vt 90° µl	Vt 0° µl	% of Vt 90°
1. Healthy	32.9	22.3	68	40.4	11.0	27
Infected	0			0		
Recovered	34.8	25.9	74	31.2	5.6	18
4. Healthy	23.7	20.4	86	28.0	14.4	51
Infected	5.0	0	0	6.0	0	0
Recovered	28.0	23.8	83		Not tested	
10. Healthy	40.9	31.7	77	40.0	25.4	63
Infected	20.9	5.8	28	29.0	0	0
Recovered	51.2	44.9	88	44.8	35.1	78
13. Healthy	33.5	18.6	55	30.4	10.0	33
Infected	0			0		
Recovered	30.5	2.3	8	32.5	11.5	35
14. Healthy	61.7	53.5	86	59.5	34.7	58
Infected	31.2	5.3	17	12.3	0	0
Recovered	62.8	32.4	52	66.6	21.6	32

DISCUSSION

In most healthy individuals the Eustachian tube can equilibrate very small pressure differences (1–5 mm Hg) between the middle ear cavity and the pharynx during deglutition (Zollner 1942 v. Dishoeck, 1947 Thomsen, 1957 Miller 1965 Ingelstedt & Jonson, 1967)

In order to imitate as closely as possible a normal tubal function all the subjects in the present investigation had to be able to equilibrate an intra tympanic pressure of 4 cm H_2O positive and negative

By measuring the air volumes passing through the tube during deglutition under a low constant overpressure the effects of different postures on the tubal ventilation could be quantitatively determined. As a mean Vt decreased by $^1/_3$ in the elevation of 30 and 20 and by as much as $^2/_3$ in the horizontal position. Corresponding effects were seen in the sitting position during inflation of a neck cuff with pressures of 15 cm H_2O and 35 cm H_2O respectively Such inflations produce the same pressures in the internal jugular veins as prevail in the elevations from 30 to 20 and in the horizontal position respectively In the lateral position the venous pressure is higher in the declive part of the head (Jonson & Rundcrantz, 1969) A tendency to greater influence on the tubal function could also be noticed if the subjects were lying laterally with the ear turned downwards than if it was turned upwards.

The fact that the Eustachian tube opens more easily to a positive than to a negative intratympanic pressure was already established by Hartmann (1870) It was lately confirmed quantitatively in the investigations of Ingelstedt & Örtegren (1963) Miller (1965) and Flisberg (1966)

It is considered most physiological to measure the tubal functi n by testing its ability to equilibrate intratympanic underpressure A positive pressure was used in the present investigation because of some observ tions in the early beginning It soon became clear during the examinations of long duration not only that Vt was smaller with negative Pm but also that the reduction of Vt in the recumbent position was so great that the tube was often locked" This did not happen with positive Pm The locking f the tube during negative Pm was suspected to be due to other factors than the exclusive effect of posture on the tubal mucosa, such as distention of mucosal vessels of the middle ear cavity particularly at the tympanic orifice of the tube Vascular distention causing congestion of the mucosa has been observed in the nose during negative transmural pressure (Ingelstedt *et al* 1969)

The postural effects on the tubal function may in reality be greater than those found in the present investigation, because under ordinary conditions *the tube has to equilibrate negative intratympanic pressures.* Pfan was equal to Pm as long as the tube was closed but when it opened during deglutition Pm decreased owing to the resistance of the measuring device

The pressure drop was related to Vr (Fig 3) When the long ear canal catheter was used (in examinations in lateral positions) the pressure drop was not more than 0.8 cm H_2O to air flows below the limit of linearity With a short catheter the corresponding pressure drop could be 0.6 cm H O as a maximum

As a matter of fact Vt exceeded 500 μl/sec. only in one subject, which means that the difference between Pfan and Pm in the other subjects did not surpass 0 4 cm H_2O But the consequences of the pressure drop if any would have caused a greater reduction of Vt in the erect than in the recum bent position as Vr was always greater in the sitting position. The percental reduction of Vt in the horizontal position would then in reality have been greater than estimated

Ingelstedt *et al* (1967) found that an increase of pressure inside the internal jugular vein caused an increase of the volume of the middle ear mucosa (Δ Vmuc) In the present investigation the values of Δ Vmuc varied according to the degree of change in position or of the neck vein compression (Fig 6) Δ Vmuc is an expression of the vascular filling of the middle ear mucosa, and a corresponding congestion of the tubal mucosa should be responsible for the changes of Vt in different positions and during neck vein compression.

Opinions differ as to whether there is any noteworthy effect on the tubal function or the tubal mucosa in different body positions or depending on changes in the venous pressure.

Thomsen (1957) examined the tubal function in a large series of normal persons, using the acoustic impedance measuring method of Metz with the subjects in the recumbent position He concluded from his results that they "might suggest that the influence of the position on the tubal function is less than previously supposed

After having studied the anatomy of the tube and found only a sparse network of vessels in the human mucosa compared to the guinea pig, Tiede mann (1965) was not convinced that variations of the venous pressure in the human tubal tissues were of any importance

The opinion of Perlman and others, however that there is an influence on the tubal function in the recumbent position and the assumption that it is caused by the increased hydrostatic venou pressure were proved by the results of the present investigation.

Functionally the Eustachian tubes and the middl ear cavities belong to th upper respiratory airways. In diseases of these airways the tubal func tion is impaired. Miller (1965) and Flisberg (1966) reported that in patients with chronic otitis media only about 40% could equilibrate a negative intra-tympanic pressure Silverstein *et al.* (1966) found a tubal dysfunction in every patient suffering from chronic serous otitis media.

Studying the Eustachian tube of human temporal bone specimens Schu-knecht & Kerr (1967) f und an increased vascularity of th mucosa and the submucosa in a tive hronic inflammation. The venous pressure increase

in the recumbent position would therefore cause greater engorgement of the tubal mucosa during inflammation than in a healthy condition. Such phenomena have been observed in the nasal mucosa (Rundcrantz, 1964 1969) and were certainly the cause of the findings in the five subjects who happened to catch colds during the course of investigation. The impairment of the tubal function was qualitatively not evident in the sitting position in three of the subjects, who could still equilibrate small overpressure although Vt was quantitatively smaller The influence on Vt in the 20 position was considerably greater and after 10 minutes in the recumbent position the tubes did not only cease opening, but back in the erect position it took between 10 and 35 minutes before the tubes recovered. This latter observation seems to be a biological phenomenon which has earlier not been noticed, but which might be an important factor in the development of middle ear diseases. The observation is also in good agreement with the clinical experience that the symptoms of acute otitis media very often start in the evening or at night when the patient has been in bed for some hours and that the symptoms are relieved if the patient gets up (Anneland, 1964)

Good tubal function is an important factor for successful hearing improvement surgery which has recently been confirmed by Holmqvist (1968) and Siedentop *et al* (1968) It is reasonable to suppose that in the post operative period non infectious inflammatory reactions of the middle ear and tubal mucosa may occur In medicine prevention is better than cure and the search for etiological factors in diseases is also the search for means of prevention The reasoning above shows that it would be logical to recommend patients with common colds, specially children with a history of recurrent acute or serous otitis media to take up a position during bed rest of not less than 20 above the horizontal plane in order to prevent tubal occlusion In the post-operative period the same advice should be given to patients submitted to tympanoplastic surgery It is also important to consider that if the patient is lying laterally the diseased ear should be turned upwards. When testing the ventilatory function of the Eustachian tube *prior to middle ear surgery* it might sometimes be a valuable complement to other methods to consider the positional influence, particularly in cases where there has lately been a mucosal inflammation

ZUSAMMENFASSUNG

Es ist seit langem bekannt dass die Durchgängigkeit der Tuba Eustachii von der Körperstellung abhängt, z. B. bei der Tuba aperta. An Personen mit normaler Tubenfunktion wurde die Durchgängigkeit der Tuba Eustachii bei verschiedenen Körperstellungen und auch bei abgestufter Abklemmung der Halsvenen quantitativ studiert. In waagrechter Körperlage oder während Drosselung der Halsvenen mit einem Druck von 35 cm H_2O wurden die Luftmengen, die während des Schluckaktes durch die Tuben *hindurchtraten um zwei Drittel* kleiner als in der senkrechten Lage ohne Drosselung. In einer schrägen Korperstellung 20 –30

über der Horizontalen war die Durchgängigkeit quantit tiv um in Drittel herabgesetzt. In der Seitenlage schien die Funktion der Tube des nach unten gerichteten Ohres am stärksten herabgesetzt. Bei erkälteten Personen hatte die Horizon tallage sehr na hteilig Folgen.

Nach klinischer Erfahrung beginnen die Symptome der akuten Mittelohrent zündung oft nachts, wenn der Patient einige Stunden im Bett gelegen hat. Patien ten mit I f ktionen der oberen Luftwege oder nach Eingriffen am Mitt lohr bei denen Dysfunktionen der Tuben zu erwarten sind, sollt n angehalten werden, k ine Körperst llung u ter 20° über der Horizo talen einzunehmen, um den nacht iligen Einfluss der waagrechten Körperstellung auf die Tubenfunktion auszuschliessen. Untersuchungen der Tubenfunktion sollten sowohl in sitzender als auch in liegender Stellung durchgeführt werden.

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COMPARATIVE STUDY ON THE AUDITORY OSSICLES RELATED TO THE PATHOGENY OF OTOSCLEROSIS

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A comparative study carried out on normal and pathologic audit ry ossicles of adult peopl revealed a particular type of macrom lecular compositi n, aggregation and st bilization, occurring in the stapedia footplat as compared to the stapedian head, malleus and incus. It i characterized by ( ) an increased solubility of the collagen fibers in solvents such as acidic buffers, as w ll as f llowing th action of hydrogen-bond and electrost tic bond breakers (*b*) a particula sensitivity to collagenase digesti n after the elimination of the siali a id. The artificial introduction f new cross-links permitted the stapedian footplate to ac quire the sam macromolecular stability the collagen of th stapedian head, incus and malleus.

The labyrinthine capsule, including also some regions of the stapedial footplate ceases to grow at the end of the second year but retains throughout life some embryonal structural characters (Bast & Anson, 1949) It was also pointed out that the fibrillary texture and the mineralization of the labyrinthine capsule varies continuously suggesting that some areas of the temporal bone are subject to constant rebuilding in adult (Engström & Röckert, 1962) However X ray cristallographic studies showed that the structure of the ossicles in non-otosclerotic cases corresponds to that of normal human bone irrespective of the ossicle concerned and of the site where the sample is removed (Meurman *et al* 1967)

Numerous attempts to correlate this embryonal structural characteristic of the adult labyrinthine capsule with the development of otosclerosis were made also th observation that otosclerotic lesi ns are frequently encountered in the stapedian footplate and rarely in the stapedian head, malleus and incus has been explained on this basis (Altmann, 1962 Chevance, 1962 Frost, 1962 Rüedi, 1963 Šercer 1961)

In an effort to clarify this problem, a comparative analytical study on the tructural stability f macromolecules and macromolecular aggregates (Vel ican, 1967 *b* Velican & Velican, 1967 *a b*) of the auditory ossicles was made.

MATERIALS AND METHODS

Serial paraffin sections, cut on 5 micra, from 22 (12 women and 10 men having an age range from 23 to 42 years) apparently normal malleus, incus and stapes were investigated The auditory ossicles were obtained 6 to 10 hours *post mortem* from patients without clinical signs of otosclerosis.

After fixation in Carnoy's solution a first lot of 11 samples of each ossicle was decalcified by treatment with a saturated solution of the di-sodium salt of ethilendiamine tetra-acetate (EDTA) buffered with sodium hydroxyde to pH 7.2 (Loe 1959) This solution has the advantage of opti mum activity with the physiological range of pH It is considered capable of preserving the integrity of macromolecular aggregates of bone collagen (Cook & Ezra-Cohn 1962) Biochemical analysis indicated that less than 1% of the hydroxyproline content of the samples was removed by the EDTA method (Glimcher & Katz, 1965) The main disadvantage of this technique is a very slow rate of action even compared with weak acids. EDTA technique did not reveal significant differences in the resistance to the process of demineralization between the middle ear ossicles, and between these ossicles and the labyrinthine capsule (Chevance 1963)

A second lot of 11 samples of each ossicle was decalcified by rapid electrolysis (1–2 hours) in a solution of 10% nitric acid and 5% trichloracetic acid, in equal quantities.

The deparaffinized sections, belonging both to EDTA and electrolysis de calcified samples were submitted to (*a*) Direct staining with Van-Gieson's picrofuchsin (McManus & Mowry 1960) (*b*) digestion with collagenase followed by picrofuchsin staining (*c*) successive digestions with testicular hyaluronidase and collagenase sialidase and collagenase lysozyme and collagenase sialidase, lysozyme and collagenase pectinase and collagenase, papain and collagenase trypsin and collagenase followed by picrofuchsin staining (*d*) treatment for 1 6 12, 24 and 48 hours at 4 C with 0 1 *M* citrate buffer pH 3.5 with urea 8 *M* in Sörensen buffer pH 7 4 with 10% NaCl followed by picrofuchsin staining (*e*) treatment with 10% formalin and 5% tannic acid in physiological saline solution and in distilled water respectively followed by citrate buffer urea, 10% NaCl and sialidase-collagenase post-treatments (*f*) toluidine blue staining ("Merck" 0 05% in citric acid–disodic phosphate buffer pH 5 0) prior and subsequent to testicular hyaluronidase and papain digestions.

Details of the methods employed in this study were given elsewhere (Velican 1965 Velican, 1968)

The following enzymes were used collagenase (Nutritional Biochem Corp) 1 mg 1 ml phosphate buffer pH 7.3 for 1 3, 6 12, and 24 hours at 37 C (control slides were incubated in phosphate buffer) trypsin (Difco) 0 1 mg 1 ml 0.05 *M* phosphate buffer pH 8 9 for 30 min at 37 C (control slides were incubated in buffer solution) papain (Difco) 2 g 100 ml 0 06 *M*



Fig. 1 ( ) Footplat f the stapes. Picrofuchsi staining following collagenase incubation the fuchsinophilic material persists. (*b*) Footplate of the stapes. Picrof chsin staini g following successi sialidase–collagenase incubations the fuchsinophilic material is completely eliminated. 770.

phosphate buffer pH 6.5 for 6 hours at 52 C (control slides were incubated in buffer solution and in papain inactivated with 10% iodoacetate) pec tinase (Nutritional Biochem. Corp) 0 8 g 100 ml acetate buffer pH 4 1 for 48 hours at 37 C (control slides were incubated in acetate buffer) lysozyme (Worthington) 5 mg 30 ml 0 02 *M* phosphate buffer pH 5.3 for 48 hours at 37 C (control slides were incubated in phosphate buffer and in lysozyme inactivated in Lugol s 1 300 buffer) sialidase (Wellcome) 1 ml 4 ml 0.2 *M* acetate buffer pH 5.2 for 24 hours at 37 C (control slides were incu bated in buffer solution) testicular hyaluronidase (Nutritional Biochem. Corp) 1 mg 1 ml 0.9% NaCl for 24 hours at 37 C (control slides were incubated in physiological saline solution and in enzymatic solution inacti-vated with 1 mg heparin per mg hyaluronidase)

The identification of collagen as a picrofuchsinophilic material was made by means of collagenase digestion and by some peculiarities revealed by polarized light (anilin and ri anol reactions)

The modifications of the normally auditory ossicles induced by our histochemical procedures were compared to the osteolytic lesions occurring in the 20 otosclerotic stapedian footplate removed surgically (age of patients rang ing between 23 to 58 years)

RESULTS

Direct picrofu hsin staining pointed out the same intensity as judged by eye, in all middle ear ossicles. D gestion w th collagenase was f llowed by a

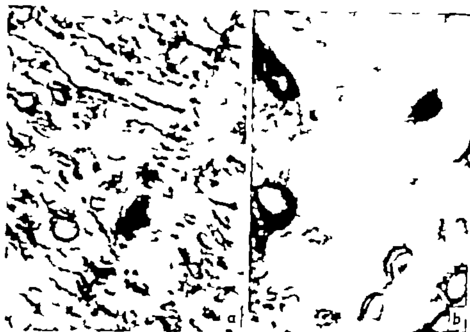


Fig 2 (*a*) Footpl t f the st pes. T l idine blue st i i g f llowing sulf tio : fibrillar struct re l revealed. (*b*) Footplat f th stapes. T luidi blue staini g bsequent t 24 hours itrat buff treatment and sulfation the fibrill struct re ca ot be revealed. 880

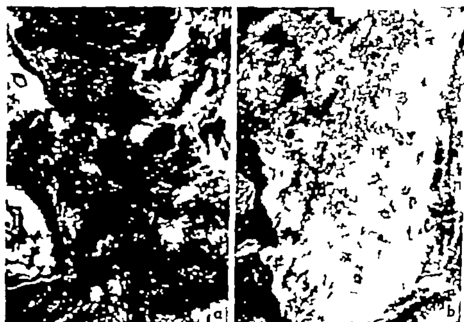


Fig 3 ( ) Head f the malleus. Picrofuchsi stai ing following 72 hours citrate buffer treatment som limited foci f collagenolysi ppeared. (*b*) Footplat f the stapes. Picrofuchsi stai ing fter 72 h urs citrat buffer treatment the collagen fibers re completely deorganized. 880.

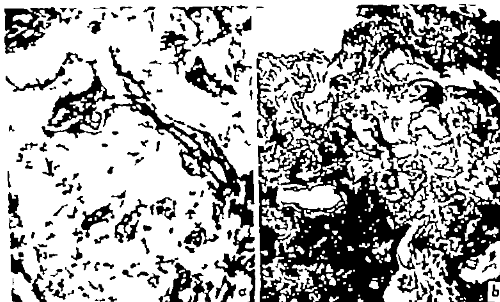


Fig 4 ( ) Footplate of the stapes. Picrofuchsin st i ing following successiv sialidase-collagenase digestion the fuchsinophilic material i complet ly liminated. (*b*) Footplat f the stapes. Picrofuchsin stai i g fter formalin pretreatment nd successi sialid se-collagenase incubations the fuchsinophilic material persists. 220



Fig 5. ( ) *Footplat of the stapes. T l idine blue stai ing revealing the metachromatic material f "unstable cartilaginou rests.* (*b*) The same microscopic field, decolourised, submitted t testicula hyal ronidase digestion nd re staining with tol idine blue the metachromatic materi l i eliminated. 220



Fig. 6 (a) Head of the stapes. Toluidine blue staining of the articular surface for articulation with the lenticular processes of the incus. (*b*) The same microscopic field, decolorized, submitted to testicular hyaluronidase digestion and re-stained with toluidine blue: the metachromatic material persists. ×220.

slight reduction of the fuchsinophilia in all these auditory ossicles. The same results were obtained subsequent to testicular hyaluronidase and collagenase, lysozyme and collagenase, pectinase and collagenase, papain and collagenase and trypsin and collagenase digestions.

Successive incubations with sialidase and collagenase determined the same slight reduction of the fuchsinophilia, except the stapedian footplate which presented a complete elimination of the reactive material (Fig. 1). Thus different susceptibility of this complex hydrolysis in the various areas of the same ossicle appeared. In the footplate of the stapes the degradation of the sialic acid permitted a collagen digestion by the specific enzyme collagenase, whereas in the head of the stapes this process could not be revealed.

Treatment with citrate buffer determined after 24 hours a weak decrease of fuchsinophilia in all auditory ossicles. In addition, a particular aspect was noted only in the stapedian footplate where the fibers which became metachromatic following sulfation and toluidine blue staining could not be revealed (Fig. 2). After 72 hours some limited foci of collagenolysis appeared in malleus and incus, whereas the stapedian footplate matrix is completely disorganized (Fig. 3).

Treatment with urea produced a slight reduction of the fuchsinophilia in all auditory ossicles; in the stapedian footplate this decrease in the staining intensity is more pronounced.

Pretreatment with formalin or tannic acid stopped the hydrolysis of the

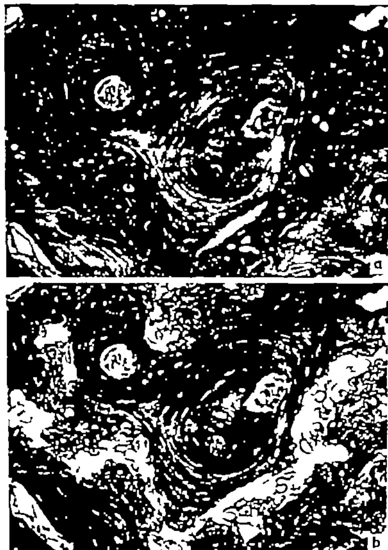


Fig 7 ( ) Incus. Acidic fuchsin-tol idi blue stai i g, revealing both the collagen fibers w ll as the "unstabl cartil gino rests. (*b*) The same microscopic field, decolouri ed, submitted t papai digestio nd re-stai ed with tol id ne blue the metachromatic material i eliminated. 220.

stapedial matrix and fibers subsequent to sialidase-collagenase digestions, as well as subsequent to citrate buffer treatment (Fig 4)

Testicular hyaluronidase digestion eliminated the toluidine blue metachromatic m terial on all un table cartilaginou rests existing in mall us, incus and stape (Fig. 5) On the contrary the metachromasia of articular surfaces of ossicles persisted (F g 6)

Papain digestion wa foll wed by the same results concerning th "un stable" cartilaginou rests (F g 7) in all audit ry ossicles. This enzymatic

action was also capable of degrading some of the chromotrope substratum existing as mucoprotein complex in the articular surfaces of malleus, incus and stapes, which was resistant to testicular hyaluronidase digestion.

No significant differences were observed, as judged by eye, between the histochemical reactivity of the material decalcified in EDTA and in acidic solutions by means of rapid electrolysis.

DISCUSSION

The results of our histochemical analysis on auditory ossicles pointed out some structural differences between the stapedian footplate on the one hand and the stapedian head, malleus and incus on the other hand. These structural differences reflect a particular type of macromolecular composition, aggregation and stabilization occurring in the stapedian footplate. As compared with the other auditory ossicles, it is characterized by (*a*) An increase solubility of the collagen fibers in solvents such as acidic buffers (solution which weaken or rupture both covalent and non-covalent linkages) as well as following the action of hydrogen bond breakers (urea 8 *M*) and electrostatic bond breakers (10% NaCl) (*b*) a particular susceptibility to collagenase digestion, but only after the elimination of sialic acid.

The solubility and enzymatic susceptibility of various collagen fractions existing in tissues were related to their degree of intramolecular and intermolecular cross-links (Gustavson 1956 Verzar 1960). Our results indicated that in the collagen of the stapedian footplate the forces between macromolecules are such that they can be overcome by various types of destroying agents. The cleavage of intermolecular linkages determined the conversion of insoluble to soluble collagen, permitting a rapid degradation of the stapedial footplate. The same destroying agents were not capable of breaking down the collagen fibers of the stapedian head or those of the malleus and incus.

Generally speaking, the bone collagen has been found to be essentially insoluble in reagents such as NaCl, urea and acidic buffers under the conditions which solubilize the collagen from a wide variety of tissues. An exception to this rule was encountered in the chicken bone collagen, where a paucity of covalently cross-linked polypeptide chains was demonstrated (Glimcher & Katz, 1965). Our results pointed out a second" exception to the rule: the human normal stapedian footplate, which presents the same apparently paucity of cross-linkages in the polypeptide chains. The artificial introduction of new cross-links (formalin and tannic acid pretreatments) was followed by a gradual increase in the macromolecular stability reflected by the resistance to specific enzymatic digestion and to acidic buffer, urea and 10% NaCl treatments. Submitted to formalin or tannic acid pretreatments which determine the introduction of numerous new cross-links, the collagen of the stapedial footplate acquires the same histochemical peculiarities than the collagen of the stapedian head and of the malleus and incus.

Our histochemical analysis revealed that the most important embryonal character which persists in the macromolecular organization of the stapedial footplate is the presence of sialic acid as a prevalent cement material of the fibers and the very slow introduction of covalent and non-covalent inter chains and intermolecular bonds in the collagen mineralized fibers. This particularity is encountered constantly in adult people who possess strong interchain and intermolecular forces in the mineralized collagen fibrils of the other auditory ossicles. On the contrary any difference appeared in the enzymatic susceptibility of the "unstable" cartilaginous rests from the adult stapedian footplate and from the malleus and incus in infants, juveniles and adults. Despite the presence of such "unstable" rests in the malleus and incus, otosclerotic lesions in these two ossicles are very rare and result by a progressive invasion starting from the labyrinthine capsule. As a consequence, malleus and incus, frequently having these cartilage rem nants, are considered unsuitable for the study of the initial stage of otosclerosis (Altmann, 1962)

A particular type of complex mucoprotein aggregation appeared in the articular cartilage of all ossicles, the strong linkages being resistant to testic ular hyaluronidase digestion, but not to papain digestion That means that chondroitin 4 and 6 sulphates are more firmly bound than the protein moiety This histochemical pattern was not mentioned in the articular carti lage of other bones and requires complementary studies.

In an attempt to correlate our observations with the pathogeny of otosclerosis, it could be suggested that the osteolytic process seems to be favoured by a sialidase-like activity associated with a collagenase one. Myxoid viruses, for instance, could elaborate a ferment which is capable of hydrolysing the cement substance of the stapedian footplate collagen fibers and the bone cells could elaborate a lysosomal hydrolase which is capal le of degrading collagen fibrils. Some results obtained in tissue culture advocated for this hypothesis (Kaufman *et al* 1965 Woods & Nichols, 1965) Thus new factors might be taken into consideration which converge in the development of otosclerotic foci.

ZUSAMMENFASSUNG

Es wurde ein vergleichende Untersuchu g ber d makromolekulare Orga isation des Steigbüg ls, Hammers und Ambosses orgenommen wobei l h n dem Steigbügel eine spezielle Art der trukturellen Stab lität gezeigt hat, die sich durch folgendes charakterisiert) l gesteigert Losll hk it der k llagenen Fibrillen l sauren Tampons, sowi in Lösungen, welch elektrostatische Verbindungen nd Hydrogenbrücken löse *b*) ei besondere Empfi dl chk it bei der Zersetzung mit K llagen se nach dem Ausscheiden d Ac dum sialicum. Durch di künstliche Einführung derer Kreuzverb d gen k die Platine des St ig bügels dens lben gehobenen Grad der strukt rellen Festigkeit wie u h die nd ren Knochenbesta dteile des Mitt lohres bekommen.

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IMPEDANCE PLETHYSMOGRAPHY ON THE VERTEBRAL ARTERY

A Possible Diagnostic Approach to the Cervical Syndrome

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Electrical impedance plethysmography has been applied to the vertebral arteries. By this method the tissue impedance which reflects volume changes, caused especially by the pulse wave, is recorded. A reduced pulsative arterial blood flow results in smaller impedance variations with each pulse wave. Special electrodes for placing against the posterior pharyngeal wall at the level of C2 have been constructed, and an attempt has been made to determine the optimal positions for skin electrodes when the head is in different positions.

In normal persons rotation of the head when in the sitting or supine position does not give rise to changes in the impedance compared with the initial positions. With the head in the hanging position, especially when it is rotated in the direction opposite to the artery being studied, a smaller impedance decrease at each pulse wave than in other positions is normally recorded. This should indicate that some compression of the vertebral artery of one side is, in a certain position of the head, a normally occurring phenomenon.

In patients with a clinical cervical syndrome, low pulse wave amplitudes simultaneous with nystagmus have been recorded. The latter can be regarded as an expression of brain stem ischaemia, caused here by compression of the vertebral artery in association with a deficient compensation via the circle of Willis.

Barré (1926) and Lieou (1928) described a symptom complex consisting of vestibular, ocular, pharyngeal and laryngeal symptoms, which they called the "syndrome sympathique cervical postérieur". Later designations are cervical migraine (Bärtschi-Rochaix, 1948), spondylotic vertebral artery compression (Sheehan *et al.* 1960), cervical syndrome (Decher 1966) and basilar artery insufficiency (Fields, 1966). The cause of the syndrome is assumed to be an intermittent ischaemia within the supply areas of the vertebral and basilar arteries.

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The syndrome is characterized by intermittent, usually mild neurological symptoms, provoked by violent rotation of the head. The most common symptoms reported are vestibular in the form a of a transient sensation of instability, sometimes combined with a tendency to propulsion and/or lateropulsion, while rotatory vertigo is more rare. Nystagmus has been recorded by Pfaltz & Richter (1958), Sandström (1961), Pirodda & Cenacchi (1965) and Decher & Sonntag (1966). Tinnitus and perceptive deafness, usually unilateral, have been reported to be common symptoms (Wildhagen, 1951, Decher 1966). The pharyngeal symptoms have been described as a sensation of a lump in the throat and transient difficulties in swallowing. Further, short periods of otalgia, hoarseness, visual disturbances, sensory disturbances in the face and also ataxia may occur. Headache has been stated to be common, mostly localized to the occipital region.

The Pathogenesis of the Cervical Syndrome

Four factors have been considered to interact in the occurrence of intermittent brain stem ischaemia.

1. *Morphological differences* in vessels and surrounding structures. Stopford (1915) found that the vertebral arteries showed the same calibre on both sides in only about 8%, while in just over 70% the calibre of the one vessel was twice as large as that of the other side, and in certain cases up to 20 times larger. Krayenbühl & Yasargil (1957), in autopsy studies, found that the arteries had an equal calibre in 26%. Kunert (1961) observed distinct calibre differences in 41%. A common finding of the above authors, and of several others, is that in cases of marked differences between the two sides the left artery is usually the widest. Hypoplastic arteries which end at the neck and thus do not contribute to the intracranial blood supply have been described (Hutchinson & Yates, 1957).

2. *Excitation of the sympathetic plexus* surrounding the vertebral artery which might possibly lead to vascular contraction with consequent circulatory impairment. At early dates Barré (1926) and Lieou (1928) considered the symptoms to be due to irritation of the sympathetic plexus around the arteries, induced by exostoses at the uncinate processes of the vertebrae. Varying information is given in the literature concerning the autonomic innervation to the vertebral artery, which forms a periarterial nerve plexus, and which has sometimes been referred to as the vertebral nerve (Wrete, 1934, Laux & Guerrier 1947, Kunert 1961).

3. *Mechanical compression* of the vertebral artery by extravascular structures, e.g. exostoses of the uncinate processes in spondylosis deformans, herniated vertebral discs or posttraumatic deformities (Virtamo & Kivalo, 1957). Exostoses have been reported to be most common between C4 and C6 (Fig. 1). As an effect of exostoses the vertebral artery can show a strikingly tortuous ("washboard") appearance at angiography (Sheehan *et al.* 1960).

The compressive effect of exostoses and similar lesions on the vertebral

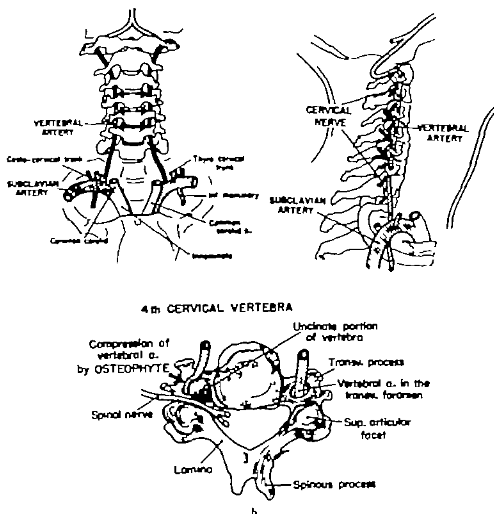


Fig 1 Frontal nd lateral diagram f course of ertebral arteries. The arrows indicat the most common sites of compression f the rtery by exostoses. The most common localization f exostoses t the base f the ncinat process (low diagram) Both arteries nd nerves ca be compressed t this sit (from Sheehan, Bauer & Meyer 1960)

artery within the transverse canal is potentiated by rotation of the head. According to several reports (Gerlach, 1884 de Kleyn & Nieuwenhuyse 1927 Primbs & Weber 1956, and others) some vascular compression occurs even normally on rotation of the head, particularly in the artery contralateral to the directi n of rotation

4 *Arteriosclerosis* of a localized or general nature in the vertebral arteries. The changes usually lie in that p rt of the vessel which passes through th transverse canal (Hutchinson & Yates, 1957 Meyer *et al.*, 1960)

It is probable that sever l of th above four factors interact to cause th cervical syndrome It would seem however that the principal prerequisite

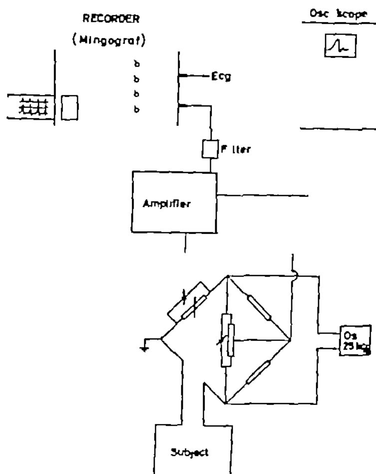


Fig 2 Principles f imped nce plethysmographi measurement of the blood fl w i bod segment. I the centre f the diagram the Wheatst ne bridge i seen (N lander *t l* 1967)

for the occurrence of the syndrome is that a unilateral constriction of the vertebral artery is not compensated immediately by adequate collateral supply from a well functioning circle of Willis.

Plethysmographic Studies of the Vertebral Artery

Attempts at functional analysis of the vertebral arteries have been made previously with the use of electrical impedance plethysmography which method was originally described for measurement of the pulse volume in extremities (Filipo, 1959 Kunert, 1961 Deidda *et al* 1963 Pratesi *et al* 1965)

The aim of the present investigation was to study blood volume variations, by means of a modified technique for electrical impedance plethysmography in association with rotation and flexion of the cervical spinal column both in normal persons and in some patients with a cervical syndrome, particu larly such patients with vertigo



Fig 3 Applicatio f pharyngeal electrodes. In *A* lectrod ha been introduced la the nose nd is tt bed firmly by suction t the post-pharyngeal wall. I *B* the other type is seen. Electrodes may be placed through both nostrils. Nea the eyes and on the forehead lectrodes are placed for rstagmography

METHODS

Definitions. Plethysmography means a recording of enlargement. The pulse wave produces volume variations in a body segment. Impedance is the resistance met by an alternating current on passing through a conductor The electrical impedance pulsation represents a changing number of ions brought to a segment by the arterial stream at a rate exceeding the venous outflow during the cycle (Nyboer 1950)

The principles of impedance measurement (Fig 2) have been described previously by Nyboer (1950) and Kaindl (1952) A four-channel oscillograph (Mingograf 42 B, ELEMA-Schönander Stockholm, Sweden) was used as recorder An increased impedance which is the result of reduced blood filling within the body segment, gives a decreased amplitude in the recordings, and vice versa. In this way a recording is obtained which at each moment gives a faithful reflection of the changes in the blood content of the tissue segment between the electrodes.

The electrode The recordings of the pulsation in the vertebral arteries were made between two internal and three external electrodes. The internal electrodes were always placed at the same position against the posterior pharyngeal wall at th level of the uvula (corresponding to C2) and were introduced via the nose after superficial anaesthesia of the nose and pharynx. Two types of pharyngeal electrodes were tested. One was in the f rm of a suction cup and w s connected to weak continuous suction in order to obtain reliable fixati n against the p teri r pharyngeal wall on rotation f the head and any swallowing movements (Fig. 3 *A*) The other one was



Fig 4 Lateral roentgenogram f cervical spinal col mn, nd showing phary geal elec trod ttached by suctio to the posteri pharyngeal wall. The positi ns of the skin lectrodes re indicated b the mbers 2, 4 6 nd 8, which represent the distance in cm t the midline t the back f the eck. The co rse f the ert bral rtery i drawn in.

placed on the end of a slightly elastic cable and was pressed against the posterior pharyngeal wall by means of a fork-shaped holder (Fig 3 *B*) The three external electrodes were placed on the skin at the back of the neck at the same level as the internal pharyngeal electrodes. A set of three skin electrodes were placed on either side of the midline. By this means measurements could be made of both the right and left vertebral arteries.

The optimal placing of the occipital electrodes was determined by means of indicators on the skin and roentgenograms (Fig 4) *It was our aim to*



Fig. 5 Contralateral rotati of the head i relatio t th artery under study The optimal positio for the skin electrod is t indicator 6, i.e. 6 cm lateral to the midline t the back of the neck.

place the pharyngeal and occipital electrodes in such a way that the artery crossed th line of communication between the electrodes For the initial position—supine position of the body with no rotation of the head—the best position for the occipital electrode was 4 cm lateral to the midline. On ipsilateral head rotation i.e rotation towards the same side as the artery under study the best positi n was 2 cm lateral to the midline On contralateral head rot tion, finally the optimal position was 6 cm lateral to the midline (Fig. 5)



Fig 4 Lateral roentgenogram of cervical spinal column, nd showing a pharyngeal lec trod tt ched by suct on t th posteri pharyngeal w ll. The position f the skin lectrodes re indicated b the mbers 2, 4, 6 nd 8, which represent the distance l cm t the midline t th ba k f the eck. The course of the ert bral artery is drawn in.

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Fig. 5. Contralateral rotation of the head in relation to the artery under study. The optimal position for the occipital electrode is located 6, i.e. 6 cm lateral to the midline at the back of the neck.

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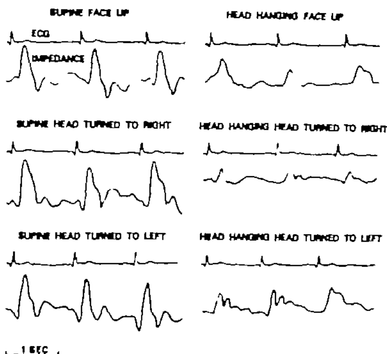


Fig 6 Impedance measurement th left vertebral art ry i normal subject. Synchronous ECG shows the relati t the impedance changes. I the supine positio the pulse wave amplit d re high. With the head hangi g, especially when iso rot ted t the right, th p lse wa e mplitud is greatly reduced and the shape of the curv ch nged.

In certain cases (upper tracings in Figs. 6 and 8) ECG was recorded syn chronously in order to see the relationship between the electrical activity of the heart and the pulse wave in the vertebral artery

RESULTS

From measurements on 15 healthy subjects it was found that high amplitudes were obtained throughout when the subjects lay relaxed in the supine position and facing upwards. Equally high amplitudes were obtained when, with the subject in the supine position, the head was turned to the right or left or flexed forwards. With the head in the hanging position the amplitudes were reduced considerably The greatest reduction in amplitude was observed with the head in the hanging position and rotated contralaterally with respect to the artery in question. A reduced pulse wave amplitude and simultaneously occurring change in the shape of the pulse curve was con sidered to be due to vascular compression

Variations in the impedance in normal persons with the head in different

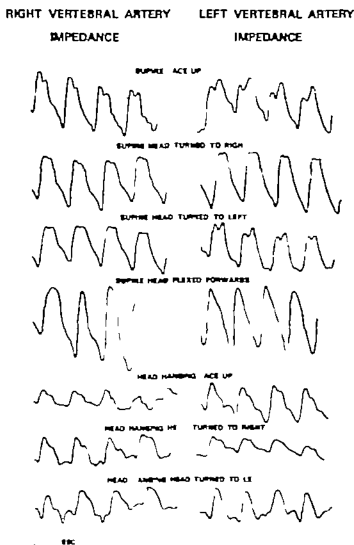


Fig Impedance measurement on the right nd the left vert bral arteries in normal subject. I the supine position w thout and with rotation f the head and with forward flexion, high-pulse w ve amplitudes re recorded. With the head ha gi g, with nd without rotation, the pulse wa mpl tudes are greatly reduced nd the shape f the curv changed.

positions are illustrated in Figs. 6 and 7 The results of measurements of the vertebral arteries on both sides are given in Fig The pattern is the same as in the preceding figure with high amplitudes in the supine position as also on rotation of the head in this body position. In this subject, forward flexion of the head led t the highest amplitudes, which together with the shape of the curve would seem to indicate that the pulse wave in this position had the least hindranc When the head was in the hanging position the

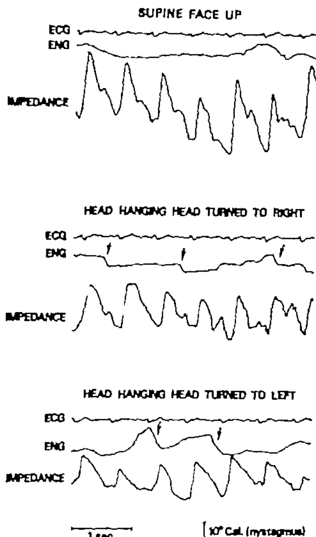


Fig. [illegible] Simultaneous electrical impedance plethysmography from the right vertebral artery, nystagmography (ENG) and ECG in patient with cervical syndrome. In the supine position, high-pulse wave amplitudes and no nystagmus were noted. With the head hanging and with simultaneous rotation to the right and left, the pulse wave amplitudes were reduced and the shape of the curve altered. In both positions of rotation 2–3 nystagmic beats (*arrows*) to the left were seen.

amplitude was reduced to half or more, particularly on rotation of the head to the right in measurements of the left vertebral artery and vice versa.

The recordings were usually preceded by local anaesthesia of the mucosa of the posterior pharyngeal wall. In three patients between 20 and 30 years of age who were just about to undergo tonsillectomy measurements were made under general anaesthesia, however. The aim here was to study whether a greater movement of the neck, obtainable in these cases, would be reflected in the impedance. No pronounced differences were noted, however, between

recordings made with local anaesthesia and those made under general anaesthesia.

Five patients with a cervical syndrome were tested. One typical case is illustrated in Fig 8 This patient was a 57 year-old man who had been troubled for a few years by vertigo of the lateropulsive type, which was provoked by backwards bending of the head. On examination an intensive, transitory nystagmus could be provoked repeatedly by bending the head backwards. No nystagmus was observed either when he was in the supine position facing upwards or when he was sitting with his head vertical The caloric reactions were normal. There was a 33 dB left-sided, neural hearing loss, while hearing in the right ear was normal. Neurological examination, including EEG, revealed an otherwise normal status. Roentgenological examination of the cervical spinal column showed spondylotic changes and disc degeneration within C5–C6 With the patient in the supine position synchronous nystagmography and recording of impedance of the vertebral artery showed no nystagmus and fairly high pulse wave amplitudes. With the head in the hanging position and with rotation both to the right and to the left left beating nystagmus and also a reduced pulse wave amplitude compared with the initial position, were recorded

DISCUSSION

Our investigations carried out hitherto indicate that the principle for impedance plethysmographic recording of pulse waves in the extremities can also be applied to the vertebral arteries. The results of measurements in normal persons have shown low pulse wave amplitudes with the head in the hanging position, both with and without rotation. A contralateral rotation in relation to the art ry being measured seemed to produce the lowest pulse wave amplitudes. These observations agree with those of de Kleyn and co-workers (de Kleyn, 1939 de Kleyn & Nieuwenhuyse, 1927) as well as with the blood flow measurements of Toole & Tucker (1960) on cadavers, and with the angiograms performed *post mortem* by Tissington-Tatlow & Bammer (1957)

Reports concerning the localization of the important sites of compression vary Both the arterial loop around the atlanto-occipital joint and the part of the artery running through th transverse canal, especially between C3 and C6, have been considered to be particularly vulnerable. With our positioning of electrodes at the level of C2, theoretically only changes up to this level could be demonstrated, but no compression peripheral to this site We therefore assumed that the reduction in the pulse wave which was manifest when the head was in certain positions was caused by compression of the artery central to the C2 level. What effect any impediment peripheral to this level has on the impedance is still unknown.

Simultaneous with the impedance measurements, nystagmography was performed (Aschan *et al* 1956) Our finding of nystagmus verifies the

results of Sandstrom (1961) and constitutes a further objective documentation of an intermittent brain stem symptom

Up to now our studies have been mainly of a methodological nature It is as yet not possible to predict whether impedance plethysmography of the vertebral arteries can have clinical applications. Since an increase of impedance occurs when the head is in certain positions even in young healthy persons, the evaluation of recordings from older persons and from patients with a cervical syndrome can be difficult In its present form the method gives a semi quantitative measure of the pulse wave and limited possibility of comparison *between different* persons.

We plan to attempt to determine whether the method can be utilized as a diagnostic aid prior to and possibly replacing the more complicated and not *completely risk-free vertebral angiography It will be of considerable value* if the method can be developed into a "screening test" in cases with assumed constriction of the vertebral artery central to the C2 level

ZUSAMMENFASSUNG

Elektrische Impedanzplethysmographie wurde an den vertebralen Arterien angewandt. Mit dieser Methode wird die Gewebsimpedanz registriert welche hauptsächlich die durch die Pulswelle hervorgerufenen Volumenveränderungen *wiedergibt Eine vermindert pulsierende arterielle Durchblutung resultiert* in kleineren Impedanzveränderungen bei jeder Pulswelle Spezielle Elektroden, die an der Pharynxhinterwand in Höhe von C2 angebracht werden können, wurden konstruiert. Es wurde auch versucht, für verschiedene Kopfhaltungen die optimale Lage der Hautelektroden zu bestimmen.

Gesunde zeigen bei Rotation des Kopfes, verglichen mit der Ausgangsstellung, weder im Sitzen noch im Liegen Änderungen der Impedanz. Bei nach hinten *herabhängendem Kopf* besonders wenn dieser in entgegengesetzter Richtung zu der zu untersuchenden Arterie gedreht wird, wird normalerweise eine kleinere Impedanzabnahme bei jeder Pulswelle als in anderen Kopfhaltungen registriert. Daraus lässt sich schliessen, dass *bei bestimmten Positionen des Kopfes eine gewisse* Kompression der vertebralen *Arterie auf einer Seite* ein normalerweise auftretendes Phänomen ist.

Bei Patienten mit Cervikalsyndrom wurden kleine Pulswellenamplituden gleichzeitig mit Nystagmus registriert. Letzteres kann als Ausdruck einer Gehirnstammischaemie angesehen werden, hervorgerufen durch eine Kompression der Vertebralarterie in Verbindung mit einer mangelnden Kompensation durch den Circulus arteriosus Willisii.

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AN INVESTIGATION OF THE HUMAN CORTICAL EVOKED POTENTIAL UNDER CONDITIONS OF MONAURAL AND BINAURAL STIMULATION

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The human cortical potential evoked by a 1000-Hz tone was recorded under conditions of binaural and monaural stimulation which covered a wide range of stimulus intensities. The results showed that (1) the curve for the amplitude as well as for the area confined by the potential increased with increases in stimulus intensity. At higher stimulus intensities, these curves leveled off. (2) Bilateral stimulation generated larger cortical potentials than did monaural stimulation. (3) Latency of the potential decreased with increases in stimulus intensity. When the monaural stimulus was delivered to the ear contralateral to the active scalp electrode, the various components of the potential showed consistently a shorter latency than when the monaural stimulus was presented ipsilaterally.

Several experiments have demonstrated that the human cortical potential evoked by sound increases in magnitude when the stimulus intensity is increased. Keidel & Spreng (1965) have shown that the growth of this potential elicited by increments in stimulus intensity within a limited intensity range can be described by a power function. Considering the fact that subjective loudness also follows a power function when plotted against stimulus intensity, it can be argued that the evoked cortical potential is somehow related to the physiological basis for loudness. This statement is made bearing in mind that integrative recordings at cortical level, as for instance slow evoked potentials, represent responses mainly to transients of the related complex stimuli. One way to provide experimental evidence directly relevant to this contention is the following. Since an auditory stimulus presented binaurally is perceived as being approximately 6 dB louder than when presented monaurally, the evoked cortical potential should be larger under conditions of binaural stimulation. Because the exponents of the power function, or the steepness of the best fitting straight line in a double-logarithmic scale, is reported to range from 0.13 to 0.23 (for a pause of 1 to 5 sec between the successive tonal stimuli), the increase of the evoked cortical potential for

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binaural stimuli would be expected to range from 0.8 dB i.e. 0.13 6 dB to 1.5 dB i.e., 0.25 6 dB Indeed, Davis & Zerlin (1966) have already reported that at least for a "comfortably loud" pip, the size of the cortical potential is larger when the listener hears the tone pip simultaneously in both ears. The experiment reported below was designed to study the influence of binaural and monaural stimulation on the human cortical potential over a wide range of stimulus intensity levels.

METHOD

Subjects

Ten listeners, comprised of students and staff members of the University of Erlangen-Nürnberg served as experimental subjects. None had a history *of chronic middle-ear disease The range of individual differences in thresh-*old for the standard stimulus used in this experiment (1000 Hz) was *restricted to 15 dB*

Equipment

Rectangular pulses recorded on tape were used as trigger for the stimulation and recording system. They were delivered simultaneously to a Computer of Average Transients, Technical Measurement Corp Model 400 B, generally referred to as a CAT computer and to a special transistorized relay switching circuit The CAT triggered a function generator (Exact Electronics, Type 255) whose frequency was set to 1000 Hz. The resulting sinusoidal signal, 800 msec in duration was delivered to a passive band pass filter (Wandel & Goltermann) whose upper and lower cut-off frequencies were 1130 and 89 Hz, respectively After the filter the signal was amplified and transmitted to an attenuator and then to a pair of matched earphones (Grundig, Model 211)

Silver-coated electrodes, 3.5 × 5 cm, were used to record the EEG associated with the tonal stimuli. Large electrodes were used in an attempt to gain a more favorable signal to-noise ratio. A differential recording arrangement was employed in which the active electrode was placed above the right mastoid process and the inactive electrode was placed on the forehead The ground electrode was located on the inion The EEG was first fed to a pre amplifier (Tektronix, Type 122) whose low-pass filter was set at 8 Hz then to another amplifier (Nagard, Type 2101) in order to attain a suitable magnitude for processing by the CAT computer The transistorized relay switching circuit, triggered by the control pulses, alternated the presentation of the tones between one earphone and load resistor and both earphones. Simultaneously the recorded EEG was alternated between channels 1 and 2 of the CAT computer For purposes of more detailed measurements, the data from the CAT computer were punched out on paper which in turn was read into a LINC-8 computer and stored on magnetic tapes. Computer programs were written which permitted rapid calculation of (1) the ampli-

index of the various components of the evoked potential, (2) the area under the curve described by the most prominent components, and (3) the distribution of remaining background EEG along various sections of the base line

Test procedure

During testing, listeners were seated comfortably in a sound-treated room. They were given shielded earphones to wear and were requested to remain quiet and relaxed during the presentation of the tonal stimuli. Every 2.5 sec a 1000-Hz tone was presented. The tone lasted 800 msec and its rise–fall time was approximately 3 msec. This was governed by the setting of the passive band-pass filter Stimulation was alternately monaural and binaural i.e., one stimulus was presented monaurally and the next binaurally This procedure prevented an influence of change of state, either physical or physiological on the EEG recording associated with monaural and with binaural stimulation. A test run consisted of 200 stimulus presentations, 100 being presented monaurally and 100 being presented binaurally The EEG responses associated with monaural stimulation were summed on one channel of the CAT computer those associated with binaural stimulation were summed on another channel.

A test session consisted of four test runs. In part I of the experiment the sound pressure levels of the tonal stimuli were presented at 50 60 70, 80 90, and 100 dB The presentation order of intensity levels within a test session followed an *ABBA* scheme where *A* represented four test runs in which the sensation levels were 100 80 60 100 dB *B* represented four test runs in which the sensation levels were 90 70 50, 90 dB Each of seven subjects were given 12 test sessions. On six of the sessions, the monaural stimuli were given to the left ear The right ear received the monaural stimulation on six sessions. The presentation order of monaural stimulation to right and left ear was balanced in a systematic manner

The plan for part II of this experiment followed that for part I The sound pressure levels, however differed. In the scheme *ABBA* *A* now represented four test runs in which the levels were 70 50 30 70 dB, respectively *B* represented four test runs in which the levels were 60 40 20 60 dB, respectively Six subjects, three of whom had participated in part I, were given 12 tests. Again monaural and binaural stimuli w re presented alternately In half of the tests the monaural timuli were presented to the left ear and in half of the tests the right ear received the monaural stimulation

Measurements

Several aspects of th complex cortical potential shown in Fig. 1 as displayed on the screen of the computer oscilloscope were measured for the response to both m naural and binaural stimulation. Number 1 in this figure represents the maximum positiv potential averaged over 12.5 msec appearing between the tim ge from 60 to 100 msec after onset of stimulation.

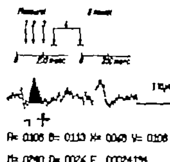


Fig. 1. Typical response pa((channel 1 f CAT 400 B Computer with monaural response l ft, channel 2 with binaural response right) on the scope f LINC-8 Computer Upward deflecti n m ns lat ral l becoming more negative with respect to the f rehead. Onset f stim is a 0 msec. *A B* (*X Y*) are the coordinates of the upper (lower) rectangula marks, which can be moved t any of the 400 addresses. *M* i the mplitude as mea valu f the curv in the rang between *A* and *B*. This val e i indicated by the right most straight line. *D* is the mean of resting background activity in th range *A–B* *F* i the rea nder the curve between the addresses *X* nd *Y* bordered by the mean value *M* (Further expl nati n see text.)

Number 2 represents the maximum negative potential averaged over 12.5 msec appearing between the time range from 100 to 140 msec after stimulus onset Number 3 represents the maximum positive potential averaged over 12.5 msec appearing in the time range between 190 to 230 msec after stimulus onset. In addition the mean value of the curve over the whole range of 190 to 230 msec was calculated. The shaded region represents the area under the curve extending from the portion of the curve connecting numbers 1 and 2 to the point represented by number 3 Number 4 represents the aver age deviation from the base line for a section of the curve extending for 37.5 msec. The record was quickly scanned and the four non-overlapping sections which possessed the least average deviation from the base line were chosen The mean of these four average deviations was calculated and this value served as the reference value to which the amplitude and area (10 times the reference) values were compared. This reference value is completely arbitrary and depends on the frequency range of the recording system and on the number of EEG responses sampled It is used for two reasons. Firstly changes in electrode resistance do not influence the final value accepted as the index for amplitude and area, since this value is essen tially a ratio, being based on the size of the fluctuation of the EEG back ground activity Secondly nearly all subjects previously tested in this Institute have shown a remarkable agreement between their subjective thresh olds and the extrapolated intensity function of the averaged evoked cortical response when the resting background EEG activity for 100 samples is used as the reference value. It is even better to attain this reference value by averaging, at the beginning and the end of each experimental session, 100 EEG samples without any stimulation

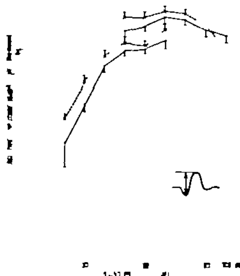


Fig 2.

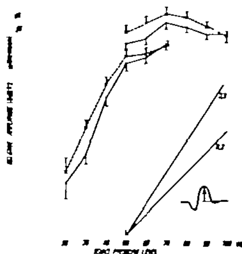


Fig 3.

Fig 2 Mean of relati amplitude (difference of amplitud t 1 nd 2 in Fig. 1) nd standard error (⌒) f the component indicated by arrows in the i set as f action of sound pressure lev L ———, m naural – – – –, binaural. (0 dB ≙ 2.5 μ\ i the mean.) The two sets f overlapping curves represent the results of the separated part I (sound pressure lev l 60 to 100 dB and part II (20 t 70 dB SPL) f the xperiment.

Fig 3 Mea f relativ amplitud (2 i Fig. 1 against mea f curv in the rang 190 to 280 msec fter stim lus onset) and standard error (⌒) for th component indicated by arrow in the inset f action of sound pressure lev L ——— Monaural – – –, binaural. (0 dB ≙ 2.5 μ\ in the mean.) The numbers nea the full-drawn straight lines indicat their steepness respectively the xponent of the pproximati g power function. The tw set of overlapping curves represent the results f the separated part I (sound pressure level 60 t 100 dB) and part II (20 t 70 dB SPL) of the experiment.

RESULTS

For purposes of illustration, the data for subjects in part I were pooled the data for subjects in part II were also pooled The means and standard deviations for the various aspects of the cortical potential evoked by binaural and monaural stimulation at each stimulus intensity level were calculated.

In Fig 2, the data for that component of the cortical potential indicated by arrows in the inset are illustrated. Log amplitude is plotted against stimulus intensity for both parts I and II What is clearly shown by this figure is that the potential behaves differently at the lower intensity range in comparison with the high intensity range Specifically from low to mode rate stimulus inten ities, th increase in amplitude followed very roughly a power function, the expon nt being approximately 0 18 Also within this intensity range binaural stimulation generated larger amplitudes than monaural stimulati n Th Wilcoxon Matched Pairs Signed Ranks test, for

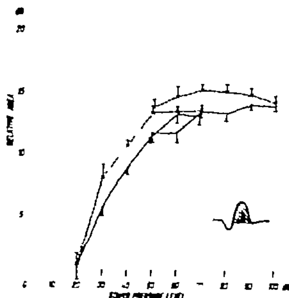


Fig. 4. Mean of relative and standard error (⌶) indicated I th. i set as a function of sound pressure level ——— Monaural ----, binaural. The two sets of overlapping curves represent the results of the separated part I (sound pressure level 50 to 100 dB) and part II (20 to 70 dB SPL) of the experiment.

example indicated that amplitudes associated with binaural stimulation were significantly greater ($p < 0.025$) than those associated with monaural stimulation at the stimulus intensities 20, 30, 50 and 60 dB. At the higher stimulus intensities on the other hand, the amplitudes failed to increase with increases in intensity. Furthermore, the differences between amplitudes generated by binaural stimulation and those generated by monaural stimulation were less.

Fig. 3 represents the amplitude changes of that component shown in the inset. The behavior of this component at the lower and higher stimulus intensity ranges is similar to that described for the component illustrated in Fig. 2. Within the lower stimulus intensity range, however, the slope of the function shown in Fig. 3 appears to be somewhat steeper. In this case a power function with an exponent of about 0.27 provides the better fit.

Area measurements followed a course similar to amplitude measurements. This can be seen by referring to Fig. 4. The area under consideration is that shown in the inset by slated lines. Like amplitude, the size of the area leveled off at the higher stimulus intensities. Binaural stimulation elicited significantly larger areas ($p < 0.025$) at the stimulus intensities 50, 0 and 80 dB.

The differential influence of binaural and monaural stimulation on the cortical potential is most clearly seen when the ratio of binaural to monaural stimulation (B/M) is plotted against stimulus intensity. Fig. 5 provides this information. Particularly with regard to amplitude, binaural stimulation

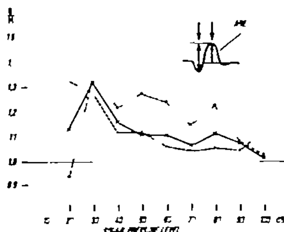


Fig. 5. Ratio *B/M* f response mplitud and rea ssociated bina ral stimulatio (*B*) t respo se amplitud ad area ssociated monaural stim latio (*M*) functio f sound pressure lev l. The mea value f the rati *B/M* i draw for the overlapping values (50, 60, 70 dB SPL) f Part I nd Part II f the experiment.

at the lower stimulus intensities consistently generated larger values than monaural stimulation. The ratio of areas of binaural to monaural responses is about 1.25 (2 dB) in the range 30 to 70 dB In the range 70 to 90 dB this ratio is about 1 15 (1.3 dB) The ratio of amplitudes over a wide range (40 to 90 dB) lies somewhat around 1 1 (1 dB)

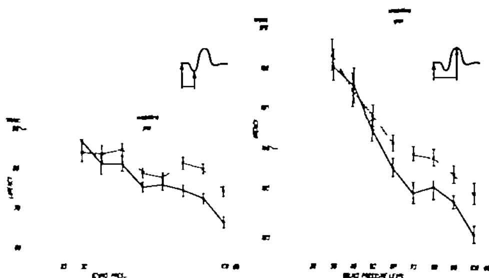


Fig. 6 Mea latency nd t ndard error (⌐) f the component indicated in the inset f nction of sound pr re l l ——— contralateral; ¬ ipsilateral. The mea l of latency i dra f the overl pp g al es (50, 60, 70 dB SPL) of Part I nd Part II of the experime t

Latency of the first positive and the first negative peak (see Fig 1) decreased with increases in intensity The rate of decrease was more rapid for the negative peak in the range 100 to 140 msec after stimulus onset Data for monaural stimulation were also analyzed with respect to which ear was stimulated What clearly emerged from this analysis was that stimulation of the ear contralateral to the active electrode evoked a potential in which the latencies of the main peaks were shorter than those resulting from ipsilateral stimulation. This differential effect of contralateral–ipsilateral stimulation on latency was greater for the negative peak (see Fig. 6) A slightly but not significantly larger amplitude was evoked by contralateral stimulation.

DISCUSSION

The data confirm the expectation that binaural stimulation would evoke a larger cortical potential than would monaural stimulation. And in so doing, the data provide further support for the notion that an increase in size of the cortical evoked potential reflects an increase in subjective loudness. But this statement holds only for low and moderate stimulus intensities up to about 70 dB SPL. Other processes must come into play at the higher stimulus intensities which counteract the excitatory effect of stimulation. And at these intensities, the size of the cortical potential fails to correspond with an increase in subjective loudness when stimulus intensity continues to increase. This does not necessarily mean that the magnitude of the potential no longer reflects loudness. Rather the interaction of excitatory and inhibitory processes may be such that the size of the resulting potential bears no direct and easily observable relation to loudness. For the overlapping values at 50 60 and 70 dB the discrepancy of the amplitudes of potentials as a measure of exitation may be caused partially by the different subjects in parts I and II of the experiment On the other hand a better agreement of this fact is shown by the area measurement the latter being much more related to the underlying change of charge in neural systems.

The amplitude function found for the lower intensity range agrees with earlier work carried out at this Institute (Keidel & Spreng, 1965 Spreng, 1967) [Because the computer enables us to chose the least average deviations from the base line as reference values all the relative amplitude values are about 1.5 times larger than former measurements, which were related to an average deviation gained by manual calculation] The slope for the component 2–3 (time range 100 to 140 msec) as plotted in Fig 3 was again found to be somewhat greater than for the component 1–2 (time range 60 to 100 msec) as plotted in Fig 2.

Latency measurements are also in line with those published by Rapin et al (1966) in that response latency was shorter when the intensity for tonal stimuli was increased Perhaps the most interesting aspect of the latency data, however is the difference resulting from contralateral and ipsilateral stimulation. In this situation, contralateral stimulation produced consistently

Table 1

Sbj.	Right dB	Left dB
Pt	115.6 ± 0.6	116.9 ± 0.9
An	113.1 ± 1.2	114.1 ± 0 6
He	114 7 ± 0.6	115.7 ± 0.9
St	116.7 ± 0.8	121.2 ± 1.2
Fl	117 1 ± 0 9	119.3 ± 0 5
Le	112.9 ± 0.7	115.7 ± 1.0
Le	114.1 ± 0.6	112.3 ± 0 7
Bu	106.8 ± 1.3	108.3 ± 1.5

Table 2

Sbj.	El. left tone right	El. right tone left
Fl	2.5	3.4
Fl	3.0	2.8
Bu	3.6	2.6
Bu	1.5	2.3
Mean	2.9 ± 0 4	2.7 ± 0.2

shorter latencies in the range 50 to 100 dB No significant difference is found *at lower sound pressure levels because of the increased variability of latency* It is true that contralateral stimulation always involved the left ear and ipsilateral stimulation involved only the right ear but it is unlikely that the difference in latency arose from the fact that the left ear or right ear *per se* was being stimulated [1] A more probable explanation for this finding is that the crossed auditory pathways offer a more direct route to the cortex than do the uncrossed pathways. And finally in agreement with the report of Price *et al* (1966) contralateral stimulation tended to provide somewhat larger response amplitudes than ipsilateral stimulation.

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ZUSAMMENFASSUNG

Akustisch usg löste Reaktionspotentiale des w chen M nschen bei Reizung mit inem Sinuston von 1000 Hz wurden unter mon urale und binauralen Reizbedingungen über einen grossen Intensitätsbereich hinweg mitt ls elektro enmaschineller Mittelung (LINC-8) quantitat untersucht. Die Ergebnisse lassen sich wi f lgt zusammenfassen 1) Amplitud und Flächenintegral der akustisch evozierten Potential nahmen mit der R izstark i sy st matischer W ise zu. Erst bei grossen

[1] contrast to the amplitud measurements—where erag value f left nd right monaural stimul tion t alw ys compared with the bina ral stim lation—for the latency measurement l ft (contra) nd right (ipsi) ear stimulation are compared. Because equal sound pressure level wa used in both ears (see Tabl 1) hearing thresholds were measured psychophysically i dB ttenuation f 10-N sin soidal ignal the earphone. A right or left dom nance ma exist i indi iduals, but measureme t tw subject on several day using tone f 70 dB nd cha ging lectrodes from left t right nd stim lation from right t l ft showed no significa t influence of dominance f ne hemisphere upon the size of evoked potentials measured in rbitrary units (T bl 2)

Reizintensitäten mündeten die Intensitätsfunktionen der Potentiale in Sättigungskurven ein 2) Bei binauraler Schallreizung waren die Reaktionspotentiale grundsätzlich grösser als bei monauraler Beschallung 3) Die Latenzen der einzelnen Anteile der evozierten Potentiale verkleinerten sich mit steigender Reizintensität. Insbesondere waren die Latenzzeiten der Potentiale bei kontralateraler Reizung signifikant kürzer als bei ipsilateraler Beschallung.

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PRESBYACUSIS

I Comparison of Manual and Automatic Thresholds

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Threshold determinations empl ying manual and autom tic audiometry were made in on experienced and on inexperienced normal control group and in two groups of presb cusis, one of which sh wed signs of acoustic trauma. The manual and utomatic tracings were superimposed in the experie ced normal group while in the other three groups the manual method resulted in poorer thresholds. Continuous and pulsed tone tracings were overlapping in the normal groups but i old ag the pulsed tone tracing was clearly better tha the steady tone threshold. The threshold amplitudes were significantly larger in presby cusi than i ormal subject the low tones in all groups showed larger amplitudes than the middle or high frequencies. Adva cing age did not decrease the performance in Békésy audiometry d the best thresholds were obtained by the utomatic pulsed technique.

The average hearing level for various frequencies as a function of age have been determined by several investigators (Bunch, 1943 Leisti, 1949 Glorig & Nixon, 1962 Corso, 1963 among others) These average curves, th ugh important in revealing the pure t ne impairment, do not bring out the main problems of old people viz. that the difficulties in understanding speech are often disproportionate to hearing levels for pure tones (Pestalozza & Shore, 1955)

In the series of experiments to be described, the psychoacoustic behavior of people with presbyacusis has been studied by various pure tone testing methods. By varying the testing arrangement and the tasks during experiments, it is attempted to find out whether pure tone testing reveals any changes possibly comparable and explanatory of the difficulties in understanding speech.

In this study the hearing level in presbyacusis were determined first by using the common manual technique. The subjects were then asked to trace the hearing levels at various frequencies with a self-recording audiometer using both interrupted and continuous test tones.

Thi study wa supported by grant from the National Research Council for Medical Sciences.

Review of Earlier Studies

Several studies have been conducted on normal adult populations to determine the manual audiometric thresholds as compared with those obtained with self recording audiometer Corso s data (1956) on 105 subjects showed that the manual method of limits produced better threshold values than the midpoint Békésy audiograms, while the peak readings were consistent with the manually obtained values at various frequencies. Later using automatic audiometry Corso & Wilson (1957) introduced further variables in testing time attenuation rate direction of frequency sweep, and tone pulsing. Increase of the attenuation rate from 1.5 to 5 0 dB/sec resulted in 5 dB better thresholds, and an increase in testing time from 2.7 to 11 0 min made the mean threshold 4 dB poorer These analyses were performed at 1000 Hz the direction of the frequency sweep and the type of the stimulus tone failed to affect the 1000 Hz threshold value For the whole frequency range tone pulsing appeared to make the threshold poorer up to 2000 Hz, but these differences were not statistically significant Above 2000 Hz tone pulsing made the mean threshold better the largest differences occurring in the 3000–4000 Hz region.

Corso s experiments were partly duplicated by Burns & Hinchcliffe (1957) in 20 subjects studied by continuous tone Békésy audiometry using an intensity change of 2 dB/sec in 8 min runs between 500–6000 Hz. Significant differences from the results with the method of limits were found only at 1000 Hz, the Békésy technique giving a better threshold by about 3 dB. This difference was attributed to the fact that the individual pure tone threshold measurements were started at 1000 Hz If this initial value was checked later during the test, the two methods gave essentially the same thresholds. The discrepancy compared with the results of Corso was attributed to the fact that while the latter instructed his subjects always to keep the tone just audible the technique of Burns & Hinchcliffe (1957) allowed the tone to fluctuate from complete inaudibility to audibility

Glorig s data from the Wisconsin State Fair reported by Rudmose (1963) indicated that the automatic self-recorded thresholds were about 5 dB better than the manual thresholds. Rodda s (1965) data, including also interoperator comparisons, suggested in a series of nine subjects, that the Békésy tracings are poorer than the manual thresholds at 250 6000 and 8000 Hz.

In the comparison of interrupted and steady tones, the repetition rates varying between 1 to 12/sec (on and off time 0 5) Palva s (1956) results showed overlapping data for repetition rates of 1 2 and 4/sec. Using rates of 8 and 12 sec the steady tone gave better thresholds this was ascribed to the fact that the duration of the pulse at these rates was insufficient for a full loudness value

Harbert *et al* (1968) studying presbyacusis, reported results in 45 cases tested by continuous and pulsed tone tracings With an attenuation rate of

5 dB/sec, the pulsed and steady tone curves were superimposed up to 2000 Hz. For frequencies of 2000 Hz and higher there was a median separation of 5 dB in favor of the pulsed tone threshold. The width of the tracings was 13 dB at lower frequencies and 11 dB for pulsed and 8 dB for steady tones at 2000 Hz and higher frequencies.

MATERIAL AND METHODS

The material consists of four groups. Normal group I consists of 19 student nurses and medical students (30 ears) between 19 and 24 years of age, normal group II of 15 outpatients (30 ears) with normal hearing, aged from 19 to 24. In group III there were 9 patients (17 ears) aged 52 to 73 years (average age 62) all having presbyacusis with a dip at 4000 Hz indicating acoustic trauma. Group IV on clinical grounds pure presbyacusis, consisted of 22 patients (39 ears) between 54 and 81 years (average age 69).

The threshold measurements were first made using the manual technique and the method of limits (Madsen Model OB 60 audiometer) with 5 dB intensity steps. Testing was started at 1000 Hz (Witting & Hughson, 1940) and the air and bone conduction tests were followed by Békésy audiometry (Grason Stadler Model E800). The thresholds were determined by the fixed frequency technique during 30 sec at each frequency first with 200 msec pulsed tones and then with a continuous tone. The tone pulse had a rise and fall time of 25 msec and an on and off ratio of 1 : 1. The intensity changes occurred in 0.25 dB steps at a rate of 2.1 dB/sec. The subjects were instructed to press the subject switch each time they heard the tone and release the switch as soon as the tone was no longer heard.

The threshold value in the manual technique was the point at which the

TABLE 1. *Average differences in thresholds obtained with manual technique and interrupted Békésy audiometry in four experimental groups*

A negative sign indicates that the manual threshold is poorer than the pulsed tone threshold.

Experimental groups	Frequency (Hz) 125	250	500	1000	2000	3000	4000	6000	8000
Normal I	5.1	2.1	−0.6	0.6	2.3	4.9	−2.5	0.6	−2.7
S.D.	7.0	6.0	5.0	5.6	7.0	4.4	5.4	6.6	6.4
Normal	−1.7	3.1	−3.3	−2.8	0.6	4.1	−4.1	−4.5	−5.5
	8.1	6.1	4.2	4.9	5.2	5.6	5.3	6.4	7.9
Dip-Group	8.4	7	−5.4	−7.8	4.4	2.8	−10.0	−5.6	−3.9
D.	6.3	6.1	0.4	3.8	6.2	7.4	6.6	6.7	6.4
Presbyacusis	5.2	−7.0	−5.6	−6.4	4.1	−1.0	−11.6	−8.1	−12.3
S.D.	9.9	9.5	6.7	7.0	7.6	7.4	7.0	9.0	11.5

Significance levels: $p < 0.01$ $p < 0.001$

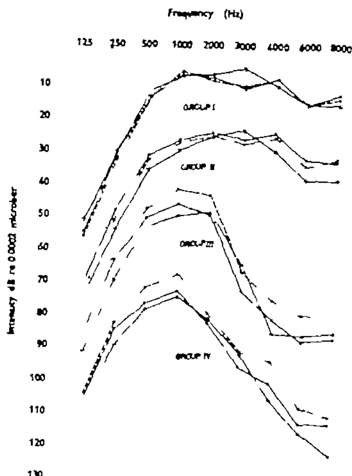


Fig 1 Comparison of manual (———) and Békésy adiometry thresh ld (continuo ●———● int rrupted t ne ●--- ●) in vari us test groups. I order to btain separati of the curves, th threshold in group II nd III re 20 dB nd those i gro p IV 40 dB larger than th real values.

patient heard 50% of the tones presented the thresholds for Békésy audiometry were calculated by computer analysis using the minimum and maximum values from which the midpoint of the excursions was obtained Calibration of the audiometer was checked before and during the period of the study

In statistical analyses the average and their standard deviations for each condition were first determined. Student s *t*-test was then employed and a level of $p \leq 0\ 01$ was taken as the minimum level for significant differences.

RESULTS

Table 1 shows a comparison of auditory thresholds obtained by the manual technique and by pulsed tone Békésy audiometry in the four experimental groups. In group I, significant differences are obtained at 125 and

TABLE 2 *Average differences in thresholds obtained with manual technique and continuous Békésy audiometry in four experimental groups*

A negative sign indicates that the manual threshold is poorer than the continuous tone threshold.

Experimental groups	Frequency (Hz) 125	250	500	1000	2000	3000	4000	6000	8000
Normal I	4.3	0.3	−2.0	−0.4	1.2	5.6	−2.0	−0.7	−3.6
s.d.	7.7	7.2	6.5	5.7	6.8	5.2	7.0	8.1	8.5
Normal II	−4.0	−5.7	−4.1	−2.8	−1.5	3.2	−5.1	−6.1	−4.8
s.d.	6.9	6.1	4.0	5.2	5.1	6.8	5.9	7.4	8.2
Dip-Group	−6.4	−6.0	−2.1	−3.0	0.9	6.5	−3.6	1.9	2.0
s.d.	6.7	6.7	8.4	5.0	7.3	6.8	4.8	7.2	6.1
Presbyacusis	−3.1	−4.7	−0.2	−1.7	−0.5	4.2	4.9	−2.8	−10.0*
s.d.	11.4	10.0	7.9	7.7	6.6	8.1	8.2	10.1	14.6

Significance levels: $p < 0.01$ $p < 0.001$

3000 Hz in favor of the manual threshold (about 5 dB) In all other groups at most frequencies, the manual threshold is significantly poorer than the pulsed tone threshold, the highest average difference −12.3 dB, being found in presbyacusis at 8000 Hz. Comparison of the groups I and II showed significant differences at 125 and 250 Hz only Similarly the results in groups III and IV overlapped and only at 8000 Hz was there a significant difference The average figures for most frequencies in group I on the one hand, and groups III and IV on the other differed significantly A similar

TABLE 3 *Average differences in thresholds obtained with interrupted and continuous Békésy audiometry in four experimental groups*

A negative sign indicates that the pulsed tone threshold is poorer than the continuous tone threshold.

Experimental groups	Frequency (Hz) 125	250	500	1000	2000	3000	4000	6000	8000
Normal I	−0.8	−1.8	−1.4	0.3	−1.1	0.7	0.5	−0.1	−0.9
s.d.	4.2	3.7	2.9	3.4	3.2	3.8	3.6	3.8	4.0
Normal II	−2.3	−2.5	−0.8	−0.1	−0.9	−0.9	−0.7	−1.6	0.7
s.	4.4	3.9	2.4	3.1	3.0	3.6	2.9	3.1	4.4
Dip-Group	2.0	1.7	3.3	4.7	5.3	5.9	6.4	7.5	5.9
s.d.	3.2	4.1	4.8	3.7	3.7	4.1	3.4	4.4	3.8
Presbyacusis	3.2	2.2	5.4	4.7	3.6	6.2	6.7	5.2	2.2
s.d.	5.9	6.3	4.8	5.8	5.7	4.2	6.2	6.1	7.9

Significance levels: $p < 0.01$ $p < 0.001$

TABLE 4 *Average excursion widths in the four experimental groups studied by interrupted and continuous Békésy audiometry*

Experimental groups	Frequency (Hz) 125	250	500	1000	2000	3000	4000	6000	8000
Normal I									
Interrupted	9	8	7	7	7	7	6	6	8
Continuous	9	8	7	7	7	7	7	7	6
Normal II									
Interrupted	11	10	8	7	8	8	8	8	8
Continuous	13	10	9	8	9	9	8	8	9
Dip-Group									
Interrupted	11	11	9	9	8	9	9	9	9
Continuous	12	12	9	8	8	7	6	7	8
Presbyacusis									
Interrupted	15	13	12	10	11	11	11	11	11
Continuous	15	13	11	11	12	11	11	12	12

but less pronounced trend was found when comparing group II with groups III and IV.

Table 2 shows a comparison of the manual technique and continuous tone Békésy audiometry. In group I, the two methods give essentially identical results, significant differences in favor of the manual method appearing only at 125 and 3000 Hz. For the normal group II the manual thresholds are significantly poorer at most frequencies. This trend continues in groups III and IV but is less marked than in group II. Significant differences between the two normal groups appeared only at 125, 250 and 6000 Hz. Comparison of group I with groups III and IV showed significant differences at 125, 250 and 8000 Hz. The inexperienced normal group and the presbyacusis dip-group differed significantly at 6000 and 8000 Hz while group II and group IV *averages showed no statistically significant difference.*

The average threshold differences between results by pulsed and continuous tone Békésy audiometry are shown in Table 3. In group I the thresholds are overlapping. The same trend continues in group II; at 125, 250 and 6000 Hz the pulsed tone threshold becomes significantly better, however. The pulsed tones give better thresholds in groups III and IV at most frequencies. The averages for both normal groups were all overlapping, which is also the case with groups III and IV. On the other hand, groups I and II differed significantly from groups III and IV at most frequencies.

The average excursion widths are shown in Table 4. Within each group, the average amplitudes for continuous or pulsed tones do not differ significantly. On the other hand, the amplitudes at 125 and 250 Hz are significantly larger than those for the other frequencies. Comparison of the four groups showed that the average threshold amplitudes were generally significantly smaller in group I and larger in group IV than in the other groups. The

results in the second and third groups were mostly overlapping and only at 250 and 4000 Hz were there significant differences among the continuous tone amplitudes.

DISCUSSION

The two control groups in this study consist of young adults. In group I they were familiar with general laboratory conditions though not with the testing procedure itself. In group II, the environment and testing procedure were novel to the subjects. The latter group is thus comparable to groups III and IV. Groups III and IV both represent presbyacusis, but the former had an acoustic trauma as an additional pathology.

In group I the thresholds with the manual and automatic technique were mostly overlapping and in agreement with the results of Burns & Hinchcliffe (1957) who found a maximum of 3 dB difference at 1000 Hz in favor of the automatic technique. In the present study the largest differences in this direction occurred at 125 and 3000 Hz (about 4 to 5 dB). The low tones, 125 and 250 Hz, however, were not tested by Burns & Hinchcliffe. These results are opposite to those of Corso (1956) who reported poorer hearing by automatic audiometry. This probably is due to the different instructions given by Corso, since his peak (minimal) values agree reasonably well with the above data.

Continuous and pulsed tone recordings were all overlapping in group I. This is in agreement with earlier data reported by Palva (1956 and 1957) and by Corso & Wilson (1957). The pulsed tone signal of 200 msec is sufficient for obtaining a full loudness value (Garner, 1947) and the minimal threshold from this point onwards is independent of the repetition rate (Rosenblith & Miller, 1949; Palva, 1956). None of the 30 sec fixed frequency tracings showed signs of adaptation. In a normal adult group, abnormal adaptation with continuous tones is so rare (Palva & Palva, 1963) that it cannot be expected to occur even though it has to be watched for.

Group II, inexperienced subjects, showed somewhat different results. The manual technique in general resulted in poorer thresholds than automatic audiometry, both for continuous and pulsed tones. Obviously, the method as such is responsible for this fact since the continuous and pulsed tone thresholds were as a rule overlapping. The differences at 125 and 250 Hz may be due to slight inaccuracies in listening at the beginning of the test, which apparently can affect particularly the continuous tracings.

The behavior of the presbyacusis-dip group and the pure presbyacusis group were basically similar. The manually recorded thresholds were clearly poorer at several frequencies than were the continuous tone thresholds by automatic audiometry, but it was for the pulsed tones that noticeably better threshold values were obtained at most frequencies. This difference clearly separates the presbyacusis groups from the two normal groups.

The separation of the pulsed and steady tone curves at various frequencies followed the same trend as observed by Harbert *et al.* (1966). However, in

the present presbyacusis group, significant differences already appeared at 500 Hz, instead of 2000 Hz, and there was no obvious increase in the separation as a function of frequency In the normal groups, the amplitude of the tracings decreased as a function of frequency a fact pointed out earlier by Palva (1956 and 1957) and by Kärjä (1968) In presbyacusis this same trend, also observed by Harbert *et al* (1966) was apparent although the present figures were somewhat larger than those of Harbert *et al* They were also significantly larger than those found in the normal groups I and II The small average amplitude of 6 dB at 4000 Hz in group III is explained by the fact that, in these ears associated with acoustic trauma, the recruitment phenomenon contributes to reduce the width of the tracings.

In normal sophisticated material it apparently makes no difference which method is used for threshold measurements. In the case of inexperienced normal listeners, the continuous and pulsed tone tracings are better than the results by the manual technique In presbyacusis cases, whether associated with acoustic trauma or not the best thresholds can be obtained by interrupted automatic recording The results by the manual technique and by continuous tone Békésy audiometry do not differ to any convincing extent

There may be several reasons for the clear separation of the continuous and pulsed tone thresholds in presbyacusis when tested by Békésy audiometry The pulsed tone is easier to listen to, and threshold determination accordingly more accurate a fact frequently mentioned by elderly patients after testing Another factor might be that continuous tone automatic recording may cause a slight amount of adaptation. Downward-sloping 30-sec recordings occurred in 3 of the 22 subjects, even when the perstimulatory threshold shift was at the maximum 5 dB. It is worth noticing, however that the switch button manipulation caused no difficulties even at advanced age 12 of the subjects were over 70 and 2 over 80 years, and their performance in Békésy audiometry was comparable to younger age groups.

ZUSAMMENFASSUNG

Bestimmungen der Hörschwelle unter Anwendung von manueller und automatischer Audiometrie wurden durchgeführt bei einer erfahrenen und einer unerfahrenen n rmalen Kontrollgruppe und bei zwei Gruppen von Altersschwerhörigen eine davon zeigte Zeichen akustischer Traumen. Die manuellen und automatischen Verfahren waren gleichwertig bei der erfahrenen normalen Gruppe, während in den anderen drei Gruppen die manuelle Methode schlechtere Hörschwellen ergab. Dauer und Impulstonverfahren griffen bei den n rmalen Gruppen ineinander uber aber bei der Gruppe der Altersschwerhörigen war die Impulston-Hörschwelle deutlich besser als die Dauerton Hörschwelle Die Hörschwellen Amplituden waren bei den Altersschwerhörigen bedeutend grösser als bei den normalen Versuchspersonen in allen Gruppen zeigten die niedrigen Töne grossere Amplituden als d e mittleren oder hohen Frequenzen Fortschreitendes Alter verminderte nicht die Leistung bei der Békésy Audiometrie und die besten Hörschwellen wurden durch utomatische pulsierende Technik erhalten

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ULTRASTRUCTURE OF THE VESTIBULAR SENSORY AREAS IN EXPERIMENTAL LATHYRISM

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The effects of β-aminopropionitrile (BAPN), a lathyrogenic substance, on the vestibular sensory areas of the rat were studied by means of electron microscopy. Degenerative changes occurred in both the type I and type II hair cells, with swelling of mitochondria, numerous large laminated inclusions, vacuolization and protrusion of the supranuclear cytoplasm. The nerve endings showed only mild degeneration. In the connective layer, numerous large membranous whorls were seen enclosing lipid droplets and smooth vesicles. It is suggested that these lesions are primarily due to a disturbance in protein metabolism. The study is a further example of ototoxicity and indicates that lesions in the vestibular end organs play a role in the pathoge esis of experimental lathyrism.

The property of inducing experimentally selective lesions in the vestibular sensory areas is limited to a small number of compounds, and their exact biochemical action is not completely understood.

It is the object of this investigation to demonstrate that, in the rat, systemic treatment with β-aminopropionitrile causes distinctive degenerative changes in the vestibular sensory epithelia, as studied by means of electron microscopy. β-Aminopropionitrile (BAPN) is a synthetic lathyrogenic compound which in various animal species reproduces experimentally the lesions known to occur after ingestion of *Lathyrus odoratus*, the flowering sweet pea. The characteristic changes are named angio-osteolathyrism and neurolathyrism. The former consists of aortic aneurysms and skeletal deformities and the latter is characterized by excitement with choreiform and circling movements ("ECC-syndrome") resembling waltzing (Selye, 1957; Gardner 1964; Bensusan, 1966). In the rat labyrinth circumscribed new bone formation in the periosteal layer (Schwetz, 1959) and ankylosis of the stapediovestibular joint (Bellucci & Wolff, 1959) have been reported, but no changes were described in the sensory epithelia.

MATERIAL AND METHODS

Twenty female albino rats of the Wistar strain with an average initial body weight of 200 g were used in this study. With the exception of six



Fig 1 Normal utricular hair cell of type I (*HC I*) and type II (*HC II*) with supporting cell (*SC*). Note multilaminated inclusion (*L*) of hair cell top. Nerve ending (*NE*). Basement membrane (*BM*) 3750

Line on this and all subsequent figures indicates 1 μ

untreated controls, the animals were injected with β-aminopropionitrile fumarate 150 mg in 1 ml of distilled water s.c. once daily. All rats were sacrificed by decapitation on the 25th day of the experiment. After dissection the ears were perfused within 5 min after death with 1% osmic acid buffered with potassium dichromate (Dalton, 1955). The specimens were dehydrated in alcohol and embedded in Epon (Luft, 1961). Serial sections were cut on a LKB Ultratome. These were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined with a Siemens Elmiskop I microscope.

FINDINGS

In the control animals no abnormal behavior or pathologic changes in the fine structure of the vestibular sensory areas occurred (Fig. 1). In all BAPN injected rats abnormal motor hyperactivity, circling and an inability to swim developed within 19 to 23 days of treatment. Morphologically distinctive lesions were produced in the vestibular sensory areas of all experimental



Fig 2 Utricular hair cells of type I (*HC I*) with nerve chalice (*CH*) and type II (*HC II*) in BAPN-intoxicated animal. Note laminated inclusions (*L*) and degeneration of mitochondria (*M*). Supporting cell cytoplasm (*SC*) bulges into endolymphatic space (*E*). It contains normal granules (*GR*) and slightly dilated Golgi apparatus (*G*). Reticular lamina (*R*), cuticle (*CU*), stereocilia (*ST*), nerve ending (*NE*). 15,000.

animals. The saccule, the utricle and the semicircular canals were equally affected, and the degenerative changes were present in both the type I and type II hair cells. None of the experimental animals died.

In the sensory cells under study protrusions of the supranuclear cytoplasm and vacuolization were common. Frequently the surface membrane was ruptured, with ejection of cytoplasmic contents into the endolymphatic space. The mitochondria were swollen, with partial or complete loss of their cristae (Figs. 2, 3 and 5). The cisternae of the Golgi apparatus were occasionally dilated. Some nuclei were pyknotic, but the nuclear membrane was



Fig 3 Degenerated horizontal ampullar hair cell of type I (*HC I*) with nerve chalice (*CH*) and type II (*HC II*). Supporting cell (*SC*) and nerve endings (*NE*) are relatively well preserved. Note vacuolization (*V*), laminated inclusions (*L*) and [illegible] of mitochondria (*M*) into endolymphatic space. Reticular lamina (*R*); cuticle (*CU*), stereocilia (*ST*); kinocilium (*K*). Golgi apparatus (*G*). 7500

unaffected. The cuticle was not affected, swelling and distortion of stereocilia was infrequent. Numerous multilaminated inclusions were found in the cytoplasm of the hair cells. They were up to 1 μ wide and 3.5 μ long and consisted of dense lines with a periodicity of 900–1400 Å. Each interval was bisected by a less dense intermediate line. This laminated structure, which was only once observed in the normal sensory epithelium of a control animal, occurred usually near the hair cell top and in relation to the cell membrane (Figs. 1–5). It was frequently associated with degenerating mitochondria, vacuolization, lipid droplets and an increase of free ribosomes



Fig. 4. Higher magnification of degenerated type I hair cell in horizontal ampulla. Laminated nucleolo (*L*) associated with vacuolization (*V*), lipid droplets (*LI*) and clusters of free ribosomes (*RI*). Granules (*GR*) in supporting cell with attached ribosomes. × 27 000.

accumulating in the surrounding cytoplasm (Fig. 4). Bulging of the cytoplasm and occasional dilation of the Golgi apparatus were also seen in the otherwise normal supporting cells (Figs. 2 and 3). The afferent and efferent nerve endings appeared relatively well preserved, and swelling of mito-



Fig. 5. Vacuolization and disintegration of cuticle-free hair cell top in degenerated horizontal ampulla. Laminated inclusions (*L*) and dilated Golgi apparatus (*G*) in hair cell. Supporting cell (*SC*), cuticle (*CU*), stereocilia (*ST*) and kinocilium (*K*) are rather well preserved. 10,000.

chondria and vacuolization were less prominent than in the hair cells. A more pronounced degeneration of mitochondria was observed in the nerve chalice of the type I hair cells (Figs. 2 and 3). The nerve fibers in the subepithelial connective membrane occasionally showed retraction of the axolemma and slightly swollen mitochondria (Figs. 6 and 8). In the connective cells and their cytoplasmic processes numerous onion-like membranous whorls occurred. They measured between 1.5 and 4.5 μ in diameter and consisted of a varying number of concentric paired lamellae enclosing lipid droplets and smooth vesicles (Figs. 6–10). The membranes of the whorls



Fig 6 Membranous whorl (*W*) i connective membrane f degenerated utricle, enclosing lipid droplet in its center Supporting cell (*SC*) Nucleus (*NU*) f connective cell. Cytoplasmic processes f connective cell (*CC*); capillary (*CA*) myelinated nerve fibers (*MY*) with slight retraction f lemma (*arrow*) non-myelinated nerve fiber (*NONMY*) intercellular substance (*IS*) basement membrane (*BM*) 7300

were often closely packed and fused in some areas. They were not continuous and usually smooth surfaced Ribosomes, if present, were mostly confined to the external surface of the membranes and to their ends peripherally (Figs. 8 and 10)

DISCUSSION

Experimental lathyrism is produced by a number of structurally similar compounds which cause different lesions in the same animal species. BAPN,



Fig. 7 Connective membrane of degenerated saccule. Membranous whorl (*W*) enclosing lipid droplets (*LI*), vesicles (*VE*), mitochondrion (*M*) and ribosomes. Nucleus (*NU*) of connective cell; myelinated nerve fiber (*MF*). × 23,000.

the compound chosen for this study is not considered to be a strong neurotoxic substance. It is better known for producing angio-osteolathyrism, a disease widely used as an experimental model in research on connective tissue diseases and aging. Various biochemical studies on angio-osteolathyrism have described a direct interaction between BAPN and protein, an increase in protein synthesis and a marked inhibition of oxygen consumption (Bensusan 1966). Protein and aminoacids supplements in the diet exerted some protective effect against angio-osteolathyrism in the rat (Gardner 1964). The neurological symptoms, on the other hand, are usually induced with iminodipropionitrile (IDPN) and occur so regularly that neurolathyrism has been recommended as an easily reproducible test object for psychotropic drugs (Thuillier & Nakajima, 1957).

In the literature the symptoms of neurolathyrism are frequently described as a "waltzing syndrome" suggesting a similarity with genetic waltzing and an origin in the vestibular system. Chou & Hartmann (1964) using rats and Slagel & Hartmann (1965) using mice demonstrated IDPN-induced axonal swelling in the anterior horns of the spinal cord and in the brain stem. The pathologic changes were prominent in axons of the lateral and medial vestibular nuclei and in the vestibulospinal tract, as well as in axons of several

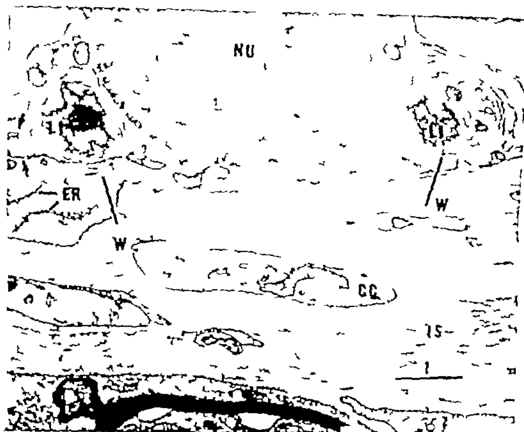


Fig. 8. Concentric membranes of degenerated utricle. Membrane whorl (W) with lipid droplets (LI) in their center. Note rough-surfaced endoplasmic reticulum (ER) and peripheral membranes of whorl (arrow) both with attachment of ribosomes to external surface. Nucleus (NU) of connective cell; cytoplasmic process of connective cell (CC); intercellular substance (IS); myelinated nerve fiber (MF). 16,250.

other cranial nerve nuclei. Ultrastructural studies of the IDPN induced lesions in the rat revealed ballooning of the axon near the perikaryon ("ghost-cells"), a decrease of ribosomes and an increase of neurofilaments and mitochondria in the axoplasm. Disturbances in protein metabolism and in axonal flow were proposed as the pathogenesis of the axonal lesions in experimental neurolathyrism (Ule 1962, Chou & Hartmann 1964). Dietzel & Ule (1963) demonstrated an increase in axoplasmic protein concentration as evidence of increased protein synthesis.

In our study of the vestibular end organs the most prominent changes occurred in the hair cell cytoplasm which showed protrusions into the endolymphatic space and contained degenerating mitochondria, clusters of free ribosomes, vacuoles and multilaminated inclusions. This BAPN induced degeneration differs from the lesion produced in the vestibular sensory cells by streptomycin ototoxicity which predominantly affects the mitochondria, the plasma membrane and the hairs (Duvall & Wersäll 1964). The multilaminated inclusions found abundantly in this experiment were first de-



Fig 9 Membranous whorl (*W*) in connective membrane of degenerated utricle (a and *b*) and saccule (c and *d*). Note lipid droplet (*LI*) smooth vesicles (*VE*) and intact mitochondria (*M*). Capillary (*CA*); intercellular substance (*IS*)



Fig 10 Membranous whorl (*W*) djacent t n cleus, i connecti cell f degenerated saccul Peripheral end f whorl, with attached ribosomes, is see between tw atact mitochondria (*M*) Not rough-surf ced endoplasmic reticulum (*ER*) and parall l layers of adj cent cisterna (*arrow*) with ttachment f ribosomes t external surface Inter cellular substance (*IS*) 27,000

scribed by Friedmann *et al* (1963) in the utricular macula of a patient with Menière s disease They were subsequently observed in normal and degenerated neuroepithelia of the labyrinth and when numerous they were assumed to be the result of a pathologic process (Friedmann, 196, Hilding *et al*, 196) Extracellularly the same laminated material was found in the subcommissural organ of the rat and interpreted as a unique type of long-spacing protein (Naumann & Wolfe 1963) Similar structures, without the

subdividing intermediate line, were observed in the lateral geniculate body (Morales *et al.*, 1964 Smith *et al.*, 1964) and in the cerebellum (Morales & Duncan, 1966) of the cat and considered as differentiations of the endoplasmic reticulum.

The presence of numerous membranous whorls in the cytoplasm of the connective cells, which was also observed in this study has not been reported previously These structures appear comparable to those produced in cochlear ganglion cells after kanamycin intoxication and interpreted as concentric lamellae of the endoplasmic reticulum (Kellerhals *et al* 1967) Similar intracellular inclusions have been described as proliferation of the smooth endoplasmic reticulum in the outer hair cells of the organ of Corti following rupture of Reissner s membrane (Duvall & Rhodes, 1967) and endolymphatic hydrops (Kimura, 1967) In our material, a relationship between the membranous whorls and the rough-surfaced endoplasmic reticulum is indicated by the occasional presence of ribosomes within the whorls (Fig 7) particularly at the periphery of the membrane (Figs 8 and 10) and by the observation of rough-surfaced endoplasmic reticulum in arrangements which suggest early stages of whorl formation (Fig. 10) Although no definite continuity between the membranous whorls and the nuclear envelope could be established, their close association in some of the cells under study is conspicuous (Figs. 7 8, and 10) Pannese (1966) has reported membranous whorls as expansive growth of the nuclear envelope in ganglionic neuroblasts, and he emphasized that the outer nuclear membrane is a cytoplasmic structure and may form cisternae of the endoplasmic reticulum. Changes in the rough surfaced endoplasmic reticulum with degranulation of its membranes and increase of smooth membranes of the endoplasmic reticulum have been reported in rat liver parenchyme cells following administration of various toxic agents, such as carbon tetrachloride (Reynolds, 1963) thiohydantoin (Herdson & Kaltenbach, 1965) phenobarbital, tolbutamide (Remmer & Merke 1965) cycloheximide (Harris *et al.*, 1968) and ethanol (Rubin & Hutterer 1968) Herdson & Kaltenbach (1965) demonstrated enzyme activity in membranous cytoplasmic whorls assumed to be derived from cisternae of the rough-surfaced endoplasmic reticulum.

The functional significance of both the multilaminated inclusions in the sensory cells and the membranous whorls in the connective cells observed in our experimental material is not established. Their abundance, however indicates that they ar due to a pathologic process. Previous interpretations of comparable cytoplasmic inclusions as being related to the endoplasmic reticulum are consistent with the hypothesis that BAPN interferes with protein synthesis Since the endoplasmic reticulum participates in protein ynthesis and changes in appearance according to the activity of the cell the multilaminated structures and membranou whorls could well be morphologic manifestations of an alteration in protein metabolism as response to a toxic cell injury

The BAPN induced morph logic lesions seen in the vestibular sensory

areas of the rat labyrinth are a further example of ototoxicity and correlate well with the symptoms of experimental neurolathyrism. Slagel & Hartmann (1965) concluded from their studies a relationship between the neurologic symptoms and the presence of axonal lesions in most parts of the central vestibular system. Our study, however, suggests that the neurotoxic effect in experimental lathyrism is due to both central and peripheral lesions of the vestibular system.

ZUSAMMENFASSUNG

Die Wirkung von β-Aminopropionitril (BAPN), einer synthetischen lathyrogenen Verbindung, auf die vestibulären Sinnesepithelien im Labyrinth der Ratte wurde elektronenmikroskopisch untersucht. Degenerative Veränderungen wurden in den Haarzellen vom Typ I und Typ II gefunden, mit zahlreichen Lamellenstrukturen, Vakuolen, Vorwölbung des supranukleären Zytoplasmas und Anschwellen der Mitochondrien. Die Nervenendungen waren weniger betroffen. In dem unter der Basalmembran liegenden Bindegewebe traten zahlreiche knäuelartig angeordnete Membranensysteme auf. Die durch BAPN erzeugten Veränderungen werden primär auf eine Störung des Eiweiss-Stoffwechsels zurückgeführt. Sie sind ein weiteres Beispiel ototoxischer Wirkung und zeigen, dass Schädigung der vestibularen Endorgane bei der Pathogenese des experimentellen Lathyrismus eine Rolle spielt.

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EYE MOVEMENTS FROM SINGLE UTRICULAR NERVE STIMULATION IN THE CAT

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Single utricular nerves were electrically stimulated in the cat. The induced eye movements were almost pure rotation in both eyes. In addition to rotation, there were upward shifts in the ipsilateral eye and downward shifts in the contralateral eye. Slight contralateral horizontal shifts occurred in both eyes. Tension increases in extraocular muscles were strongest in the ipsilateral superior oblique and contralateral inferior oblique. They were weaker in the ipsilateral superior rectus and contralateral inferior rectus, and were weakest in the ipsilateral medial rectus and contralateral lateral rectus muscles. The tension increase in the contralateral inferior oblique and the lateral rectus was studied by changing the frequency of stimulation. The utricular–ocular reflex reacted from very low to very high frequencies, as high as 1600 cps. In comparison, the cupulo-ocular reflex arc did not respond well to high frequencies. The temporal summation in the vestibulo-ocular reflexes appeared different between the utricular, the vertical ampullary and the lateral ampullary systems.

By selective stimulation of single ampullary nerves in cats, eye movements induced by individual semicircular canals were demonstrated in our previous reports. Although there are a few similar studies on the effects of utricular nerve stimulation, it is not clear just what the pattern of eye movement is from each utricle. This is a report of such a study. The utricular nerves were stimulated using a similar technique as for ampullary nerve stimulation. The object of this study was to demonstrate what eye movements are produced by a single utricle and whether or not they are different from those induced by the canal nerves.

METHODS

About 50 cats were used in this study. Under ether anesthesia the spinal cord was cut between the C_1 and C_2 levels. The subsequent ear surgery was

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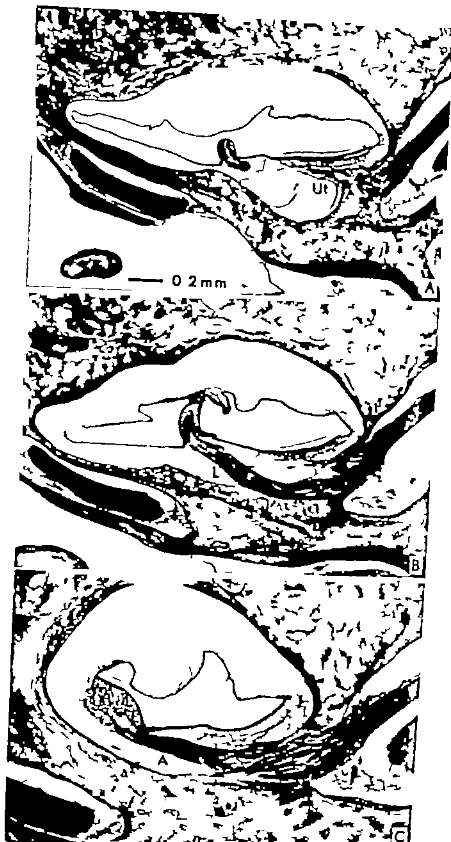
This paper was read at the 19th Meeting of the Japan Society of Vestibular Research held in Tokyo, 1966, and at the 17th General Assembly of the Japan Medical Congress, in Nagoya, 1967.



Fig. 1. Lateral-ventral view of the left inner-ear of the cat. *Ut* indicates the utricular nerve, *A* the anterior canal nerve, *L* the lateral canal nerve, and *S* the saccul

conducted under local anesthesia and the animal was maintained under artificial respiration. During the experiment cats were usually sleeping and generally did not show any signs of pain. If they did, the experiments were continued by adding local anesthesia or under light general anesthesia with Nembutal. Active eye movements were thus maintained as a guide for implanting the electrodes at each of the individual branches of the vestibular nerve. The electric field generated at the tip of the bipolar electrodes was so restricted that their implantation was possible only by observing eye movements induced by electric stimulation characteristic to each of the three ampullary and utricular nerves.

Enamel-covered 60 μ stainless steel wires were glued together with vinyl solution to make a bipolar stimulating electrode. It was cut flush at the tip. One of the electrodes was inserted through the oval window and placed under direct vision at the utricular nerve (Figs. 1 and 2). The oval window was packed with a piece of soft tissue and the area was covered with paraffin to protect the window and then fixed with acrylic. Implantation of the


Ut
0 2mm
A
L
B
A
C

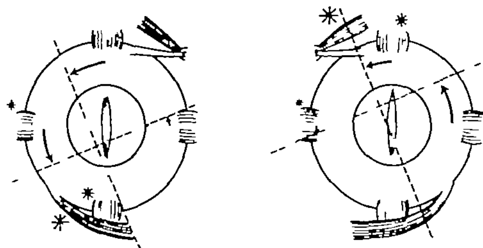


Fig. 3. Diagramatic illustration of utricular activity in the eye muscles and movements in the cat. When the left utricular nerve was stimulated, the eyes moved in the direction indicated by the arrows. The asterisks of different sizes indicate the different magnitude of evoked activities; the largest, the most active, etc. The induced movements of the eyes are accordingly counterclockwise in both eyes with components of ipsilateral up-contralateral down- and bilateral horizontal shifts.

electrodes at the ampullary nerves was done by using techniques described in our previous paper (Cohen *et al.*, 1964 and 1965 Suzuki *et al* 1964)

Individual nerves were electrically stimulated with square waves of 0.1–0.5 msec duration. They were usually given in trains which consisted of varying numbers of pulses between 2–50 separated by 0.6–25 msec. For studying the responses induced by different frequencies of pulses, the number of pulses in the trains was first set constant and only pulse separation was changed. Accordingly the pulse trains with shorter pulse separations had a shorter length, and those with longer pulse separation, a longer length.

Eye movements induced by pulse trains were recorded by cinematography for closer observation, and analyzed by recording the tension of the individual extraocular muscles which were activated. Techniques of recording isometric muscular tension change are the same as in the previous reports (Cohen *et al* 1964 and 1965) Electromyography was also used to compare the results with those obtained by recording muscular tension.

Fig. 2. (*A–C*) Histological section with stain showing the dimensional separation of the utricular lateral and anterior ampullary nerves in the vestibule. These three sections are separated by 200 μ. *U L* and *A* indicate the utricular, lateral and anterior ampullary nerves respectively.

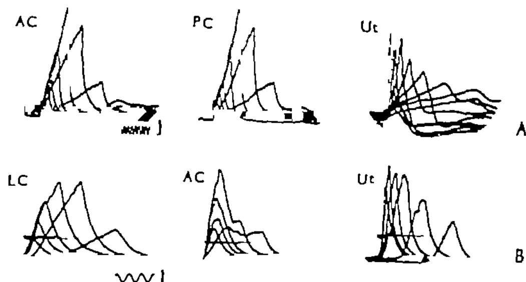


Fig 5 (*A* *B*) Eye muscl contraction from mpullary nd utricular nerve stim lation with trains with varied pulse separati l th alert nd Nembutalized cat. I (*A*) re shown superimposed traces f eye muscle contraction (the right l ferio blique in *AC* nd the right inferior rect s in *PC* and *Ut*) activated by trains f 30 pulses with p lse separation f 1.0, 1 6, 2.5 4.0, 6.4, 10.0, 16.0, 25.0 msec These were pplied t the nteri canal (*AC*) f the al rt cat. The l st h extra trace fo a p lse train with 33 msec separation. Notice that, in *AC* and *PC*, the lopes of contracti re on the same line f from 1.0 through 6.4 msec f p lse separati In *Ut* they re t, the slope of 1.0 msec i the steepest. In (*B*) re shown uperimposed traces f muscl co tractions in a cat under light Nembutal nesthesia. Pulse separations were 0.6, 1.0, 1.6, 2.5, 4 0 and 6.0 msec nd the trai was applied t the lateral (*LC*) nterior (*AC*) canal nd utricula (*Ut*) nerves on th left sid Recordings were tak from the right l t ral rectus for *LC* nd from th right l ferio blique for *AC* a d *Ut*. Calibratio re 20 cps nd 5 gms for each of the top nd the bottom traces.

nerve stimulation the maximum slope is the same for from 1 0 through 6.4 msec of pulse separation while, in utricular stimulation the slope of the contraction for 1 0 msec of pulse separation, i.e. 1000 cps is the steepest.

The patterns of the response from stimulation of vertical canal nerves were similar to each other and somewhat different either from the pattern of responses of the utricular nerve or of the lateral canal nerve The differences were sometimes exaggerated under light Nembutal anesthesia (Fig. 5 *B*) The slope of muscular contraction from lateral and anterior ampullary nerve stimulation was the same for pulse separation of from 0 6 through 2.5 msec while that for 0 6 was the steepest for utricular stimulation. Thus under Nembutal frequency characteristics of utricular nerve responses were still maintained emphasizing the much higher rate of responses as compared to canal responses.

Fig 6 *A* and *B* shows EMG recordings which demonstrate the differences in responses of the primary eye muscles activated by utricular anterior and lateral canal nerve stimulation Responses produced by trains of double

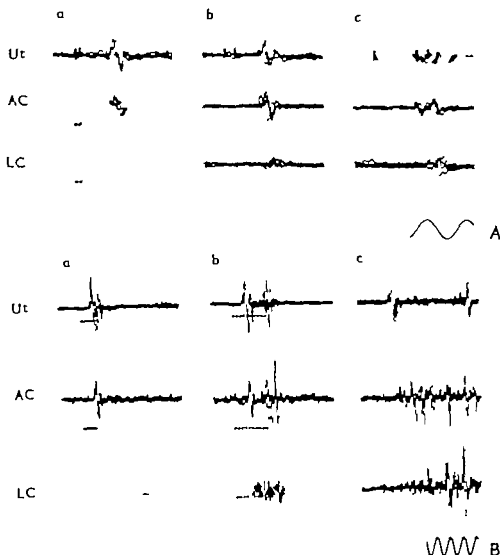


Fig 6 (A B) EMG recordi g f ey m scles acti ted b utricula nterio canal nd lateral canal nerv stim lation. I (A) doubl p lses were pplied t the utricula (*Ut*) anterior canal (AC) nd lateral canal (*LC*) nerves on the left side The m scles from which EMG recordi g w taken were the i ferior blique for *Ut* nd *AC*, nd the lateral rect for *LC*. I (*B*) t p lses were pplied i st ad. Pulse separation w 1 msec in ( ) 2.5 msec i (*b*) nd 6 4 msec ( )

pulses are shown in Fig 6 A EMG pot ntial from the utricle were largest when pulses of 1 msec separation were used while those from the lateral canal were largest w th pulse separati n of 6 4 msec. Those from the anterior canal w r termediate Similar tendencies were shown when the pulse trains had t n p lses in tead of two (Fig 6 B)

In ddition F g 6 *B* hows the pattern of response build-up produced by

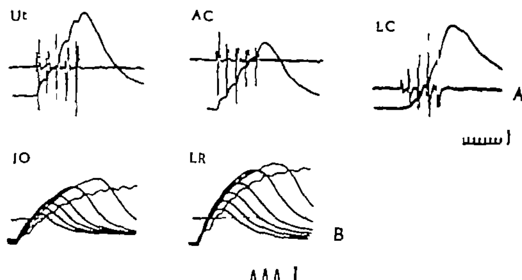


Fig. 7 (A, B). Simultaneous recordings of eye muscle tension and EMG of the eye muscles activated by single utricular and ampullary nerve stimulation in the alert cat (*A*) and eye muscle contraction from motor nerve stimulation (*B*). (*A*) Five pulses with 16 msec pulse separation were applied to the utricular (*Ut*), anterior canal (*AC*) and lateral canal (*LC*) nerves. Tension recording and EMG were taken from the inferior oblique for *Ut* and *AC* and lateral rectus for *LC*. Calibrations are 100 cps and 2.5 gms. (*B*) Superimposed eye muscle contraction traces were obtained by applying pulse trains of 20 pulses to the motor nerves of the inferior oblique (*IO*) and the lateral rectus (*LR*). Pulse separation were 0.6, 1.0, 1.6, 2.5, 4.0, 6.4, 10.0 msec. Calibrations are 100 cps and 2 gms.

recurrent stimulation of the nerves. When lateral canal nerve were stimulated, small potentials were provoked by trains of 1 msec pulse separation, but potentials provoked by trains with a 6.4 msec pulse separation showed a smoothly recruiting build up and attained the largest potential toward the end of the train. In contrast of this, during utricular nerve stimulation largest potentials were provoked by trains of 1 msec pulse separation and the potentials provoked by trains with 6.4 msec separation were smaller and frequently showed inhibition in the midst of responses as shown here. The anterior canal responses were again intermediate in characteristics between lateral canal and utricular responses.

In Fig. 7 *A*, simultaneous recordings of EMG and muscular tension show the differences between the responses from the utricular and the semicircular canal nerves. The smoothly recruiting EMG potential increase in the lateral rectus was reflected in the smooth convex curve of the tension increase of the muscle. These tension increases have been analyzed in detail in a previous study (Suzuki & Cohen, 1966). The utricular and anterior ampullary nerve responses, on the other hand, were characteristic in having a large initial potential followed by irregular-sized responses in the EMG. These were reflected more or less in the irregular tension increases which accompanied them.

These differences were not due to the differences in the muscles them-

selves which were activated Thus, as seen in Figs. 5 and 6, the differences between utricular and anterior canal nerve responses were demonstrated in the same muscle, the contralateral inferior oblique. However the biggest difference was seen between lateral ampullary and utricular responses. These have different primary muscles of their own, the contralateral lateral rectus for the former the contralateral inferior oblique for the latter The motor nerves innervating these muscles were stimulated. As seen in Fig. 7 *B* both muscles reacted similarly to motor nerve stimulation.

DISCUSSION AND COMMENT

The function of both the otolithic-ocular and the cupulo-ocular reflexes is to provide ocular compensation for head movement. Thus, head-tilt to the side in the coronal plane induces torsional eye movements. These tend to maintain the initial position of the eyes as long as the head is tilted The torsional motion is due to otolithic reflexes, and is called compensatory counter rotation of the eyes. The two vertical semicircular canals on one side are also capable of inducing rotatory movements of the eyes during rotation of the head in the coronal plane.

Thus the utricle and the vertical canals must work together in inducing a similar type of eye movement during head-tilt to the side. The utricle reacts mainly to the direction change of the head with respect to gravity and the canals react mainly against acceleratory rotation of the head. From these compensatory movements it might be predicted that the eye movements from utricular and those from combined vertical ampullary stimulations would be exactly the same The present experiment, however reveals that small differences exist between these movements. The utricle produces, while the vertical canals do not produce, a horizontal shift of the eyes although this is small in magnitude compared with the rotatory components which are the main part of the response

It should be emphasized first that because of the physical separation of the vertical canal nerves, it is not likely that an electric current delivered through an electrode implanted at the utricular nerve would spread to both the anterior and posterior canal nerves at the same time.

The spread of stimuli to either or both of the lateral and the anterior canal nerves from the utricular nerve electrode however should be considered as a possibility To avoid the current spread, the electrode must be implanted at the nerve as far as possible from the point of their bifurcation. An electrode can be implanted close to any two or three of the lateral ampullary anterior ampullary and utricular nerves, and then even a low intensity stimulation can induce mixed responses. When the electrode is placed close to only one of these nerves, however current spread would not occur even with a stimulation at considerably higher voltages, 2–4 times of the threshold stimulus.

The contralateral horizontal shift of the eyes from utricular nerve stimula-

tion could be due to the current spread to the lateral canal nerve. This possibility was excluded however by showing that temporal summation from the utricle was different from that from the lateral canal. The study will be reported in the following paper.

The contralateral horizontal shift of both eyes, although it is not large in amplitude, may be related to positional nystagmus of direction changing type which is typically caused by alcohol intoxication and is also frequently encountered in clinical cases with infratentorial lesions. In a preceding paper we showed experimental data which suggested that the otolithic organs, probably the utricle induces the slow phase of the direction changing positional nystagmus in the horizontal plane. In normal subjects in normal conditions, however, otolithic influences are not so strong as to let the induced eye deviation become large enough so as to cause nystagmus (Suzuki *et al.* 1968).

There has been discussion on whether nystagmus is induced from the utricle or not. We demonstrated that nystagmus was induced by stimulating the nerve with recurrent pulses with rather higher voltages. The direction of the quick phase of nystagmus was in the opposite of that described above.

According to Szentágothai who reported on eye movements induced by stimulating the utricle mechanically and electrically, there were four different eye movements from each of the four quadrants of the single utricular macula (Szentágothai, 1964). In our study it is likely that all nerve fibers of the nerve branch were stimulated as a whole although one portion of the nerve might be stimulated more effectively than the rest. In these studies we produced only one predominant pattern, horizontal rotatory eye movements with intorsion of the ipsilateral eye and extorsion of the contralateral eye. The vertical components varied somewhat. These differences may be due to the differences in the location of the stimulating electrode.

According to anatomical studies of the utricular macula by Flock (1965) in fishes all the hair cells of the utricle are lined up in a radial fashion, the central part of which is in a caudal lateral direction. In the lateral canal crista the stereo-kinocilial direction is related to the direction of induced eye movements. If it is also the case with the utricle, eye movements from the utricular macula can be predicted by summating the effects from stimulation of these individual cells. Electric pulse stimulation utilized in the present report may have activated all or a part of nerve fibers in the utricular nerve. The eye movements thus induced will be then a summation of those responses from individual fibers. It may be then quite reasonable that the utricle induces torsional eye movements.

For interpretation of our results using electric pulses, we should consider one of the most important differences between electric pulse stimulation and the natural stimulation. Natural action potentials induced by head tilt or head rotation must be "desynchronized" rather than "synchronized" as is the case when the nerves were electrically stimulated. To test responses of the animal at different frequencies of stimulation, we used pulse trains with

a set number of pulses in the train. This was done to maximize the response to frequency as expressed in terms of rate of tension increase. Rate of tension increase is synonymous with "eye-speed" and is regarded as the best indicator of the intensity of the responses (Cohen *et al* 1965 Suzuki & Cohen 1966)

When using trains with a fixed number of pulses, the present study showed that the utricular system has remarkably high frequency characteristics as compared to those induced by canal nerve stimulation. As reported in previous studies, spontaneous discharges of individual nerve fibers of otolithic nerves as well as of ampullary nerves were around or even below 100 cps. Our stimulus frequency went much higher than this and the maximum was even around 1600 cps.

Stimulation of the nerves with a pulse interval of 1 msec was definitely more effective than that with longer intervals, for example, 2 msec, for the utricular nerve but it was not the case with the vertical or the lateral canal nerves. The nerves in the utricular system must have, therefore, EPSPs of very short time constant in order to react to those high frequencies of stimulation.

The lateral canal system was hardly excited with short pulse trains. A steady recruitment of responses during repetitive pulses is however most pronounced with the lateral canal nerve stimulation (unpublished data) It is less pronounced with vertical canal, and much less, with utricular nerve stimulation (Fig 6 *B*) Lateral canal nerve stimulation with longer pulse trains accordingly induces stronger responses than the others, especially the utri ular system. This may indicate that the former has many collateral recruiting pathways compared to the others. Also the utricular system appears to have stronger inhibitory feed-back than the others.

The significance of these differences are important since the differences must be directly connected with the differences in the neuronal characteristics and neuronal networks as well. The neuronal and synaptic characteristics should have significant physiological or functional as well as neurophysiological implications, although they are not clarified yet. The answer will probably be given at least partially by exploring potentials in the brain stem.

ZUSAMMENFASSUNG

Einige N. utriculus der K tze wurden l ktrisch stimuliert. Di induzierten Augenbewegungen waren fast rei rotat risch auf den beiden Augen. Zugleich zeigte sich Aufwartsverschiebung des psilateralen Auges d di U terwärts- erschiebung des co tralateralen Auges. Ausserdem f nden sich die geringgradigen horizontalen Gegenwärtsverschiebungen in beiden Augen. Di induzierte Tensi s-zunahme der äusseren Augenmuskeln wa am stärksten in dem ipsil teralen M. obliquus superior u d i d m contralateralen M. bliquus inferior schwacher i dem ipsilateralen M rectus superi und dem ontralateralen M. rectus inferior

und am schwächsten in dem ipsilateralen M. rectus medialis und in dem contralateralen M. rectus lateralis. Die Tensionsannahme des gegenseitigen M. obliquus inferior und M. rectus lateralis wurde durch die Anwendung der verschiedenen Frequenz der Reizung registriert. Der Utriculookularreflex reagierte auf den Reizen von Niederfrequenz bis zur Hochfrequenz, sogar 1 600 Hz. Der Cupulookularreflexbogen reagierte auf solchen Hochfrequenzen des Reizes nicht. Die zeitliche Summation des Vestibulookularreflexes trat verschiedentlich zwischen den utriculären, den vertikalen ampullären und dem lateralen ampullären System auf.

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NASOTRACHEAL INTUBATION IN ACUTE EPIGLOTTITIS

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P inting out the serious course, with rapidly progressing airway obstruction, of acute epiglottitis in children, the authors submit a material of ight patients, seven of whom were hildren. Treatment was by antibiotics and prolonged nasotracheal intubation using tubes of polyvinyl chl ride. The intubation proved easy to establish. Complications during and afte the intubation were few and negligible, nd most of the hildren could be extubated within th first 48 hours. The critical period in cute epiglottitis is the first 24 hours. Thereafter th oedema rapidly subsides, the epiglottis having returned to an entirely normal state on the 5th–8th day Since all the patients could be discharged as cured within 2 weeks, we feel that in future nasotracheal intubation should be preferred t tracheotomy I the treatment of acute epiglottitis.

Since Sinclair in 1941 described acute epiglottitis (a.e) as localized in flammation of the epiglottis of acute onset and causing severe airway obstruction, there have been several reports stressing the rapid course, the serious prognosis, and the therapeutic possibilities (Gasser 1952 Berenberg & Kevy 1958 Vetto, 1960 Rosales & Davenport, 1962 Vainer & Ludvigsen, 1962)

In most cases the disease develops within 24 hours. The first signs are a sore throat and elevated temperature, followed in a few hours by respiratory difficulty in the form of unrest, air hunger stridor with retractions in the jugulum, and cyanosis which untreated may lead to death by suffocation, especially in children

Among 11 cases of a.e that came to autopsy in the Institute of Forensic Medicine Copenhagen, because of sudden, unexplained death, Poulsen & Simonsen (1964) found the average duration of the disease to have been 20 hours.

The rapid onset of the symptoms is due to acute, inflammatory oedema of the epiglottis which quickly swells to fill more or less, the aditus laryngis, so that total obstruction may result In some cases there will be development of an abscess which may perforate spontaneously

The aetiology of the disease has not been finally elucidated. Minor lesions of the epiglottis ha e been mentioned as predisposing factors (Vainer & Ludvigsen, 1962) Others have suggested a possible relationship between chronic tonsillitis and a.e (Kyttä, 1960 Neuberger & Moritsch 1956 Triff

& Bak Pedersen, 1968) The predominant bacterium found is Haemophilus influenzae, type B (Sinclair 1941 Berenberg & Kevy 1958) but other bacteria too have been demonstrated. Beckmann (1963) mentioned virus as a causative factor

The differential diagnostic possibilities are mainly acute laryngitis stridulans (pseudocroup) foreign bodies in the larynx or trachea, allergic oedema of the larynx, or retropharyngeal abscess, but the diagnosis is easy to make by indirect laryngoscopy which shows a red, swollen usually "cherry like" epiglottis.

Treatment consists partly in relieving the upper airway obstruction and partly in fighting the infection with antibiotics So far the most common treatment of severe airway obstruction has been tracheotomy previously done under local analgesia, but recently most often under general anaesthesia.

In Gasser's material of 25 children aged 1–7 years 5 were not tracheotomized, and of these 4 died. Tracheotomy was done immediately at admission on 14 patients, 2 of whom died Of the 4 patients who had tracheotomy more than 2 hours after admission 3 died. Three patients died during the tracheotomy procedure. Vetto's material comprised 37 children, 2 of whom died immediately after admission Twelve were tracheotomized, and of them one died a few hours later Vainer & Ludvigsen's (1962) material of 90 patients included 17 children, 11 of whom were tracheotomized, and no deaths occurred among the children. Rosales & Davenport (1962) reported on 51 children with a.e., 90% of whom were tracheotomized immediately under intubation anaesthesia, and they had no deaths. In Träff & Bak Pedersen's (1968) series of 22 patients 12 were children tracheotomy was done on 9 children, while 2 were treated by prolonged nasotracheal intubation No deaths occurred.

As is apparent from the above tracheotomy has saved the lives of many children, but nevertheless several have succumbed in spite of the tracheotomy three even during the procedure All authors agree that the prognosis of a.e is best when tracheotomy is performed as early as possible Recent results (Rosales & Davenport 1962 Träff & Bak Pedersen 1968) show that the tracheotomy should be carried out under intubation anaesthesia. Since tracheotomy in children may give rise to complications, during and after the procedure as well as in the course of decannulation, prolonged naso- or orotracheal intubation has recently been introduced in several clinics (Brandstater 1962 Allen & Steven, 1965) The incidence of complications from the upper air passages has been considerably reduced by the advent of polyvinyl chloride tubes for the intubation. Polyvinyl is an ideal material for tracheal tubes due to its physical properties, *int al* of becoming soft at body temperature while retaining its lumen and adapting itself of the surroundings in the nose and larynx, its smooth surface and its biological inactivity Since the duration of the epiglottic oedema, and thus also the danger of obstruction, is relatively short-lasting in a.e we have introduced prolonged nasotracheal

intubation instead of tracheotomy The object of the present paper is to demonstrate the applicability of this treatment

MATERIAL, METHOD AND RESULTS

During the period June 1967 to September 1968 we have treated eight patients with a.e Of these patients seven were children aged 1–5 years and one was an adult (Fig 1) All the children exhibited the classical picture, starting as a sore throat, dysphagia and elevation of temperature up to around 39 C. The respiratory difficulty developed 4–11 hours after the onset of the disease and within a maximum of 16 hours all the children had been admitted with respiratory difficulty inspiratory stridor stagnation of secretion, and in some of the patients also cyanosis and unmistakable cerebral affection, in the form of anxiety unrest, weakness, and a blurred sensorium. The diagnosis was made by the otologist immediately on admission. The epiglottis was found to be red and swollen, filling more or less the aditus laryngis. One patient exhibited signs of incipient abscess formation The respiratory difficulty was so serious that two of the children had to be intubated immediately and two within one hour while the last three were closely followed before the intubation which took place up to 3½ hours after admission (Fig 1)

All the children had nasotracheal intubation, using smooth polyvinyl chloride tubes of the make Portex or Rüsch. The intubation might be imaged to be difficult because of the oedematous epiglottis covering the aditus laryngis and the vocal cords. Owing to the dysphagia there were, besides, invariably ample quantities of secretion in the hypopharynx. However by direct laryngoscopy using a Macintosh laryngoscope, and by simultaneous compression of the oedematous epiglottis by the tube, it was possible to pass the tube into the larynx without major difficulty if necessary by the aid of Magill s forceps. All but the youngest child, who was collapsed and was only treated with oxygen, were intubated under inhalation anaesthesia with halothane (Fluothane®) and nitrous oxide Some children had premedication by atropine intravenously before the anaesthesia. After the intubation the patients were placed in an Air Shield croupette with oxygen humidification, and Alevaire® Antibiotics were administered, usually penicillin and tetracycline in large doses. Three of the children were nourished intravenously during the first days, while the others were able to take fluids by mouth. All the children tolerated the tube well, only a few needing sedatives in low doses. No complications occurred during the period of intubation. However the youngest patient had to be extubated because the tube had been occluded by secretion. Re-intubation was not necessary

The duration of intubation was from 22 to 83 hours (Fig. 1) We aimed at rapid extubation which was carried out when the epiglottic oedema began subsiding After the extubation, however reddening and moderate swelling of the epiglottis persisted fo a few days, and the normal state was not

AGE/SEX (YRS)	DURATION OF SYMPTOMS BEFORE ADMISSION (HOURS)	DURATION OF RESPIRATORY DIFFICULTY BEFORE ADMISSION (HOURS)	TIME OF INTUBATION (HOURS AFTER ADMISSION)	DURATION OF INTUBATION (HOURS)	SYMPTOMS AFTER EXTUBATION	EPIGLOTTIS NORMAL (DAYS AFTER ADMISSION)	DURATION OF STAY IN HOSPITAL (DAYS)
1¼ ♀	8	8	IMMEDIATELY	22	STRIDOR FOR ½ HOUR	7	11
2½ ♂	7	3	1	39	SLIGHT HOARSENESS FOR 24 H S.	7	9
3 ♂	18	8	2½	30	INTERM TTENT STRIDOR FOR 24 HRS	7	10
3½ ♀	15	4	IMMEDIATELY	23	—	6	8
4 ♂	13	4	3½	38	—	8	11
4½ ♂	8	6	½	47	HOARSENESS FOR 48 HRS	6	11
5 ♂	6	3	1½	83	NTERM TTENT STRIDOR FOR 4 DAYS	7	14
25 ♀	24	—	NOT INTUBATED	—	—	4	8

Fig 1 Durati f symptoms, treatment by intubation, and co rse f acut epiglottitis.

attained until 6–8 days after admission (Fig 1) After the extubation three patients had slight stridor one because of subglottic oedema caused by too large a tube and perhaps too prolonged intubation. The vocal cords were entirely normal in all cases. Four exhibited small pulmonary infiltrations already at admission, a complication to the disease, not to the intubation The infiltrations subsided in a few days. On the 4th–5th day the temperature was normal, and the children could be discharged, completely fit, from the 8th–14th day (Fig 1)

In the adult patient intubation was not needed, as there was no respiratory difficulty

DISCUSSION AND CONCLUSION

The incidence of acute epiglottitis in children appears to have been on the increase in recent years (Träff & Bak Pedersen, 1968) This is confirmed by the material submitted here which comprises 7 children and only one adult patient

The present study has brought no further elucidation of the aetiology Bacteriological study from the epiglottis, done in 4 cases, showed Haemophilus influenzae in one, pneumococci in one and a normal pharyngeal flora in the remaining two Our material clearly illustrates the extremely rapid course of the disease and the rapid onset of respiratory difficulty in children (Fig 1) Although all the patients were presented at an early stage for correct treatment in hospital, the youngest one had collapsed because of pronounced hypoxia at admission. In such very severe cases there have been

reports on cardiac arrest which has been successfully treated with external cardiac massage and artificial ventilation (Rosales & Davenport, 1962)

Cortisone and antihistamines have been tried by several workers, but we do not feel it is permissible to await their doubtful effect in such debilitated patients. In very debilitated patients the intubation may be performed without anaesthesia preceded by artificial ventilation with oxygen, but in most cases inhalation anaesthesia is preferable. Muscle relaxants should not be used because the resulting apnoea cannot be treated by artificial ventilation as intubation is impossible due to epiglottic oedema. The psychic trauma of prolonged intubation in the waking child is definitely not worse than that of tracheotomy. Every day we have been able to perform indirect laryngoscopy with good cooperation in most of the children.

Humidifying the inspired air is even more important in prolonged intubation than in tracheotomy as the tubes have a smaller lumen than the corresponding tracheotomy tubes and as the nasotracheal tube is also appreciably longer with a consequently greater tendency to stagnation of secretion and crust formation in the tube. However, we did not have major secretion problems with the use of the croupette, obstruction of the tube occurring only in the youngest child.

In frequent indirect laryngoscopies we did not observe ulcerations of the oedematous epiglottis or abscess formation caused by the tube. The oedema started subsiding already after the first 24 hours. Therefore extubation could be done in six children within the first 48 hours, and stridor following extubation was minimal. Thus, several factors indicate that the airway obstruction in acute epiglottitis in children passes its most severe phase in the course of the first 24 hours, when treated quickly and energetically with antibiotics. Thereafter the oedema and airway obstruction rapidly subside. Accordingly the object of the treatment must be to abolish the airway obstruction in the first 24 hours. The present nasotracheal intubation, therefore possesses several advantages over tracheotomy which in acute epiglottitis is an operation on a patient in a very poor condition.

ZUSAMMENFASSUNG

Bei 8 Patienten mit Epiglottitis acuta, wobei es sich um 7 Kinder handelt, wird der ernste Verlauf mit schnell zunehmender Obstruktion der Luftwege sowie die Behandlung mit Antibiotika und prolongierter nasotrachealer Intubation mit Polyvinylchloridtuben beschrieben. Die Intubation wurde leicht durchgeführt. Während und nach der Intubation sind nur wenige und unbedeutende Komplikationen registriert. Die meisten Kinder konnten schon während der ersten 48 Stunden extubiert werden. Bei Epiglottitis acuta ist die kritische Periode während der ersten 24 Stunden, später nimmt die Epiglottisschwellung schnell ab, und die Epiglottis ist schon vom 3. bis 8. Tag wieder normal. Weil alle Patienten schon nach höchst 14 Tagen gesund entlassen werden konnten, meinen wir dass die nasotracheale Intubation bei Epiglottitis acuta der Tracheotomie vorzuziehen ist.

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THE ROLE OF THE PARASYMPATHETIC AND SYMPATHETIC INNERVATION FOR THE SECRETION OF HUMAN PAROTID AND SUBMANDIBULAR GLANDS

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Human parotid and submandibular glands have been studied histochemically and ultrastructurally with the aim to achieve a morphological basis for establishing the relative importance for salivary secretion of the parasympathetic and sympathetic nervous system. The two glands are innervated by parasympathetic and sympathetic nerves, which are no doubt, both of functional importance. The secretion which remain after damage of the parasympathetic nerves, due to surgical trauma or pathological conditions, is in all probability mediated largely by sympathetic nervous activity.

It is well-known that salivary secretion is controlled via the autonomic nervous system but the relative importance of the sympathetic and parasympathetic nerves has not been assessed. However, it has been reported that section of the chorda tympani, or of the glossopharyngeal nerve in man, reduces the submandibular and parotid secretion (Enfors, 1962, among others). These nerves contain the parasympathetic secretory fibres to the submandibular and parotid gland respectively. The sympathetic nerves, on the other hand, are seldom damaged and owing to their anatomical localization they are also difficult to reach for experimental studies. Therefore the largest interest in the literature has been focused on the parasympathetic innervation of human salivary glands, while relatively few studies have been devoted to the sympathetic innervation.

During the recent years, new histochemical and electron microscopical methods have been developed, which permit the selective and specific demonstration of the sympathetic (adrenergic) and parasympathetic (cholinergic) nerves. Such methods have been used in the present study on human parotid and submandibular glands.

MATERIAL AND METHODS

Tissue was obtained from non neoplastic parotid and submandibular glands, in seven cases at parotidectomy and in seven cases at extirpation of submandibular glands.

Catecholamine fluorescence

Small pieces of tissue were taken for freeze-drying and treated with formaldehyde vapour for the visualization of adrenergic nerve terminals (Falck, 1962 Falck *et al* 1962) Paraffin sections were studied with a fluorescence microscope with routine filter equipment (Falck & Owman, 1965)

Cholinesterase staining

Cryostat sections were stained for acetylcholinesterase according to two modifications of the original method of Koelle & Friedenwald (1949) (Gomori, 1952 Holmstedt 1957) using Mipafox as an inhibitor of non-specific cholinesterase and an incubation time of generally 45 minutes

Electron microscopy

Small pieces of tissue were immediately immersed in ice-cold 3 per cent potassium permanganate solution (Richardson, 1966) and fixed for 30 to 90 min (Hokfelt, 1968) The tissues were rinsed in Ringer solution and contrasted *en bloc* in a 1 per cent uranylacetate solution, and finally dehydrated in ethanol and embedded in Epon (Luft 1961) Ultrathin sections were cut on an LKB-ultratome and sometimes contrasted by lead citrate (Reynolds, 1963) The sections were examined in an RCA EMU-3A or a Philips 300 electron microscope

RESULTS

Flourescence microscopy (Fig. 1)

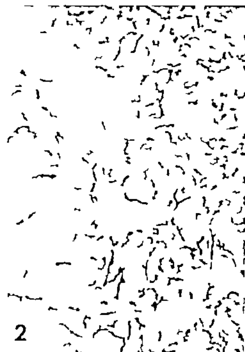
The acini in both the submandibular and parotid glands were surrounded by bundles of nerve fibres, exhibiting the intense fluorescence and abundant varicosities typical of sympathetic adrenergic nerve terminals They were in close contact with the basal parts of the acinar cells but did not extend between the cells. Each bundle could often be observed to contain several fluorescent terminals. No adrenergic innervation was found around larger excretory ducts In the neighbourhood of the ducts there could be observed bundles of non-terminal adrenergic axons, but they were not in close connection with the ducts. Blood vessels of various size in both glands were supplied with adrenergic nerves arranged in the typical way as a network on the outside of the smooth muscle layer of the tunica media.

Fig 1 Huma parotid gland. The adrenergic nerv terminal f rm a network nclosi g the cini but not the excret ry duct (*a*) Fluorescence microscopy 130.

Fig 2 H ma submandibula gland. A network of holi ergic nerv terminal ncl se a large part of the ci i. Cholinest rase stai ing. Phase co trast. 130.

Fig 3 Human submand b la gl nd. A bundl f axons surro nded by Schw cell ( ) The majority f the (*b*) in thi b ndl contai granul esicles nd probably represent cholinergi ons. One on (*a*) cont i s predomina tl small granula vesicles characteristic of drenergic ons. Note the comparati ely l rge extra cell la space ( ) separating xo from the parenchym l cells (*p*) 35,000.




2

Cholinesterase staining (Fig. 2)

A network of cholinesterase positive nerve terminals was found surrounding the acini of the submandibular and parotid glands. A few fibres were sometimes observed in the neighbourhood of the excretory ducts. The blood vessels were entirely devoid of cholinesterase-positive innervation. It could often be seen that the cholinesterase-positive nerve terminals had a varicose appearance, similar to that observed in the fluorescence microscope for the sympathetic nerves.

In some cases, the number of nerves was found to be somewhat larger in the submandibular than in the parotid glands, but often no apparent difference could be established. Generally, more sympathetic adrenergic terminals were observed than parasympathetic fibres revealed by cholinesterase staining.

Ultrastructure (Fig. 3)

Bundles of nerves, generally containing one to five axons, partly or completely surrounded by a Schwann cell were found between the parenchymal cells. Axonal enlargements being completely free of the Schwann cell cytoplasm were sometimes seen. The nerve bundles were observed in close relation to acinar cells, striated and intercalated ducts and myoepithelial cells. They were sometimes separated from the acini by processes of fibrocytes, and they often ran at a rather long distance from the parenchymal cells. Sometimes, however, no tissue was seen between nerves and glandular cells. So far, the nerves have not been observed to penetrate the basal membrane of the acinar or myoepithelial cells, and no distinct so-called synaptic contacts were seen between nerves and parenchymal or muscle cells.

With the present technique it was possible to distinguish between two types of axon containing two types of intraneuronal vesicle. These vesicles were found in the highest numbers in the axonal enlargements, the so-called varicosities, although they were also found in the thin axons. One type of axon (sympathetic adrenergic) contained small and large granular vesicles with diameters of about 500 and 1000 Å respectively, the smaller (noradrenaline storage granules) generally occurring in greater numbers. Another type (parasympathetic cholinergic) contained *agranular* vesicles only, predominantly with a diameter of about 500 Å. The two types of axon were generally found in the same bundle, enclosed within the same Schwann cell.

DISCUSSION

Until recently there has been a lack of specific methods for the morphological study of autonomic neuro-effector relations. It has therefore been difficult to relate functional data (see Babkin 1950, Burgen & Emmelin 1961) to a sound structural basis. In recent years, the fluorescence histo-

chemical method of Falck and Hillarp (Falck, 1962 Falck *et al.*, 1962) has provided a possibility to visualize the adrenergic transmitter noradrenaline within the sympathetic adrenergic neuron Staining for acetylcholinesterase permits a tracing of parasympathetic neurons, but the specificity of this method is not as well established as that of the fluorescence method mentioned

Fluorescence microscopy has revealed, in the rat, a rich adrenergic ground plexus of nerve terminals around the serous acini of the submandibular and parotid glands, while few terminals were found in the sublingual gland (Norberg & Olson, 1965) The histochemical and ultrastructural aspects of the autonomic innervation of human salivary glands have been reported recently (Garrett, 1967 Norberg *et al* 1969) Both salivary glands studied, the submandibular and parotid, are thus supplied with a rich network of sympathetic and parasympathetic nerves. The light microscopical observations indicate that they provide a double innervation of the acinar cells, and possibly also of the myoepithelial cells.

The technique used for electron microscopy i.e. the type of fixative used (potassium permanganate) permits a distinction between adrenergic and cholinergic axons (for references see Hökfelt, 1967 1968) which is often difficult when other fixatives are used (Nilsson, 1964 Hökfelt & Nilsson, 1965) It could be shown that both types of axon often run within the cytoplasm of the same Schwann cell, thus reaching the same effector cells The ultrastructural observations thus corroborate the light microscopical results indicating that the parenchymal cells receive a double innervation from both sympathetic and parasympathetic systems.

In some tissues, such as the *central nervous system (see Hökfelt, 1968)* and the sheep lacrimal gland (Yamauchi & Burnstock, 1967) so-called close synaptic contacts have been found, where the distance between nerve and effector cell is only about 200 Å Such close contacts have not been found in certain other tissues, such as the rat iris (Hökfelt & Nilsson, 1965) and were not observed in the present material In the latter cases the distance between nerve and effector cells is rarely below 1000 Å. The transmitter substance thus has to pass a larger distance in these cases, in order to reach the effector cells

The present histochemical results indicate that the human submandibular and parotid glands are reached by fairly equal numbers of sympathetic and parasympathetic nerves, the former sometimes being more common. This is in discordance with earlier reports, indicating a predominance for parasympathetic nerves (see Rauch, 1959) However it cannot be excluded that some of the parasympathetic nerves have escaped detection, owing to an incomplete staining

Previous functional data support the view that the parasympathetic nerves are the main secretory nerves to the salivary glands. Since these nerves reach the submandibular and sublingual glands via the chorda tympani, they are open to injury in pathological conditions such as chronic otitis and idio-

pathic facial paresis, and as a result of trauma, i.e. skull fractures (Magielski & Blatt, 1958), radical operation for chronic otitis (Molin & Profazio 1957) and surgery of the stapes (Bull 1965, Wiberg, 1969). In these conditions, there can often be found a reduced salivary secretion, obviously owing to damage of parasympathetic secretory nerves.

It has not been established to what extent the remaining secretion is dependent on the sympathetic innervation or humoral factors. The occurrence in the human salivary glands of large numbers of sympathetic adrenergic terminals of a typical appearance, reaching the acinar cells, would indicate that they are of functional importance. In the dog, it has been shown (Shimamoto & Inone 1958) that secretion of the submandibular gland as a result of parasympathetic nerve activity was inhibited by the i.v. administration of adrenaline or noradrenaline, or by concomitant stimulation of the sympathetic nerves, but such treatment or stimulation alone cause on the contrary a sparse secretion. If the salivary glands in the man have the same type of innervation as in dog—and this seems very probable in view of observations on several other mammals (Norberg & Olson, unpublished observations)—it can be inferred that the remaining secretion after damage to the parasympathetic nerves, in all probability is mediated at least largely by sympathetic nervous activity.

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ZUSAMMENFASSUNG

Glandula parotis und Glandula submandibularis des Menschen hat man histochemisch und elektronenmikroskopisch untersucht. Die Absicht war eine morphologische Basis zu erhalten für die Beurteilung der relativen Bedeutung der Speichelsekretion von dem parasympatischen und sympatischen Nervensystem. Die betreffenden Drüsen werden von den parasympatischen und sympatischen Nerven innerviert, welche beide zweifellos von funktioneller Bedeutung sind. Die Sekretion, die nach Beschädigung der parasympatischen Nerven durch chirurgische Verletzungen oder pathologische Verhältnisse übrigbleibt, wird wahrscheinlich zum grössten Teil von der Aktivität des sympatischen Nervensystems verursacht.

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ULTRASTRUCTURE OF THE HUMAN TYMPANIC MEMBRANE

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In order to arrive at a better understanding of the aetiology and pathogenesis of middle-ear diseases through basic knowledge concerning the electron microscopic appearances in the normal middle ear a study of the ultrastructure of the human tympanic cavity has been instituted in our Laboratory The preparations for the present study were fixed from 55 min to 4 hours *post mortem* Fixation later than 3 hours *post mortem* resulted in structural autolytic damage The tympanic membrane consists *of three layers the epidermis whose ultrastructure was found to corre*spond to the findings in the epidermis of normal skin, the lamina propria containing loose connective tissue, vessels, nerves, and two layers of collagen fibrils (also in the pars flaccida) and the lamina mucosa. In the pars flaccida the mucosal layer consisted of simple squamous cells with microvilli All through the pars tensa there were cells of varying height, often pseudostratified columnar cells with a large number of cilia and secretory granules. Goblet cells were not found in the normal tympanic membrane, but in large numbers in two cases of chronic otitis media. *Under pathological circumstances the faintly secreting ciliated* epithelium of the drum may be converted into a briskly secreting epithelium.

To this very day the aetiology and pathogenesis of several middle-ear diseases remain unelucidated. This applies especially to serous otitis media, chronic otitis media, and cholesteatosis. *One possibility of approaching the* solution of these problems is submicroscopic study—morphological as well as histochemical—of the appearances in these diseases. If the result of such a study is to be utilized, it is of decisive importance to possess basic knowledge of the normal ultrastructure in the tympanic and adjacent cavities, the auditory tube, and tympanic membrane Such an investigation has previously been performed on animals (Lim *et al* 1967 Lim, 1968) but not on human temporal bones which are, therefore now being studied in our Laboratory

Light Microscopic Structure of the Tympanic Membrane

The tympanic membrane consists of three layers epidermis, lamina propria and mucosa

The epidermis is made up of low stratified, squamous epithelium without papillae, corium hairs, or glands (Bloom & Fawcett, 1962 Beck, 1965) Normally the horny cells are not desquamated, but wander to the periphery in a process called migration. This phenomenon was first described by Burnett (1877) who noticed that a foreign body in the tympanic membrane moved towards the periphery This observation has later been confirmed and investigated more in detail (Stinson, 1936 Litton, 1963 Alberti, 1964 Franz, 1966) These studies revealed that the epithelium migrated from the umbo radially towards the periphery at an average rate of 0 05 mm/24 hours. Growth of epithelium from the auditory canal over the drum was never observed

The lamina propria is composed of connective tissue with arteries, veins, lymphatics, and nerves. The latter are present both with and without myelin sheaths and end in a terminal net in relation to the fibrous fibres in the pars tensa as well as in atypical, subepithelial Meissner corpuscles (Hirsch 1928 Riegele 1933 Andrzejewski, 19 4) Within the lamina propria there are collagen and possibly a few elastic fibres. Several workers have found the lamina propria to be absent at the site of the pars flaccida (Eggston & Wolff 1947 Bloom & Fawcett 1962) Marx (1935) demonstrated lamina propria in the pars flaccida in about half of the studied drums. The collagen fibres may be divided into an outer thick radial layer and an inner layer which contains circular parabolic and transverse fibres (Secondi, 1951 Werner 1960)

The mucosa is usually described as consisting of simple squamous cells (Eckert Möbius, 1926 Marx, 1935 Bloom & Fawcett 1962 Beck, 1965 Lim 1968) Buch (1967) found in several sites of the tympanic cavity pseudostratified columnar cells, but at the site of the drum only simple squamous cells. Sade (1966) found cilia in several sites of the tympanic cavity but never on the drum

MATERIAL AND METHOD

From 55 min to 4 hours *post mortem* 6.5% glutaraldehyde was instilled through the tympanic membrane into the middle ear of adult patients without clinical signs of tympanic or middle-ear disease 10–24 hours later the temporal bones were removed and transferred to a sucrose-cacodylate buffer in which they were stored at +2 C for a period ranging from 11 days to 12 months. Thereafter the tympanic membrane was dissected under a stereomicroscope Suitable pieces of the pars flaccida as well as the anterior and posterior half of the pars tensa were post fixed in cold 2% buffered osmium tetroxide for 2 hours (Palade 1952) In a few cases the anterior and posterior halves of the pars tensa were further divided into a central and peripheral part After step-wise dehydration, the preparation was embedded, via propylene oxide in Epon 812 (Luft, 1961) For light microscopy sections of 1 μ stained with 1% toluidine blue were used Thin sections were



Fig. 1 Micrograph of the epidermis. Cell membranes are observed in the stratum corneum. Keratohyaline granules (*KG*) in stratum granulosum, and tonofibrils (*Tf*) in stratum spinosum are noted. Marked interdigitation of cell membrane in stratum spinosum. Desmosomes (*D*)

cut on an LKB ultratome and Reichert OM U2. After staining with saturated uranyl acetate and/or lead citrate (Reynolds, 1963) the sections were studied in a Philips electron microscope EM 100 B using magnifications of from 4000 to 120 000

In two cases the tympanic membrane was fixed in osmium tetroxide immediately after removal during a radical ear operation done because of chronic otitis media. These preparations were included in the material to ascertain by comparison whether the other preparations of the material had undergone ultrastructural, autolytic changes because of too late a fixation

RESULTS

Epidermis

The epidermis consists of (1) the stratum corneum, (2) stratum granulosum (3) stratum spinosum, and (4) stratum basale.

Stratum corneum

The stratum corneum is the end product of epidermal differentiation It proved difficult to preserve the full thickness of the stratum corneum during sectioning. Cell membranes and desmosomes could be identified (Fig 1) but cell organelles were absent The cell layers numbered from one up to 6–7

Stratum granulosum

This layer was composed of 1–3 layers of cells. Several keratohyaline granules of irregular shape and size were seen both isolated and accumulated around tonofibrils. In addition, numerous lamellar granules were observed (Fig 2 *A*) but otherwise only a few cell inclusions. The cell borders were relatively smooth with numerous desmosomes, but without the marked interdigitation of the cell membranes found in the stratum spinosum and stratum basale.

Stratum spinosum

This was characterized by numerous tonofilaments, usually grouped into tonofibrils. They were interspersed with numerous mitochondria, while otherwise the cytoplasm was filled with ribosomes (Fig. 2 *B*) In most cases the stratum spinosum comprised only 2 or 3 layers of cells.

Stratum basale

This consisted of a single layer of cells with a high nucleo-cytoplasmic rati In shape they were polyhedral or elongated and arranged parallel to the basal lamina. Several cytoplasmic projections of the basal cells extended into the dermis. In addition to mitochondria, endoplasmic reticulum, ribosomes, and tonofibrils, half desmosomes were observed along the basal lamina (Fig 2 *C*) Melanin particles were not observed.

Lamina Propria

The predominant structure of the lamina propria was the collagen fibrils. As a rule the more pronounced, lateral and radial layer of fibrils was in direct contact with the epidermal lamina basalis (Fig 3) At times, there would be interposed a thin subepidermal layer of loose connective tissue Further medially there were circular parabolic and transverse fibres, more scattered and cut at different angles. Towards the medial side of the drum the connective tis ue became looser and at this site there were in addition



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Fig 2 (*A*) Strat m corneum nd stratum gra losum with les (*N*) kerat h line gra l (*KG*) t fibril (*Tf*) nd lam lla gra les (*arrows*) (*B*) Stratum sp os m with t nofilament gro ped int t nofibril (*Tf*) several mitochondria (*M*) nd band t ribosomes the cytopl sma. (*C*) Electronmicrograph showi g strat m basal (*SB*) separated from underlying l mina propria (*LP*) b basal l mina (*BL*) Sever l half desmosomes re noted (*arrows*)




LP
0.5μ



Fig 3 Epidermis (stratum corneum in upper left corner) and part of lamina propria. Immediately below epidermis the radial collagenous bundles (*RC*) are seen in cross-sectional view.

to fibroblasts and macrophages, also nerve fibres (most unmyelinated) and numerous capillaries. This layer bordered on the mucosa by a basal lamina. *Neither vessels nor nerves were seen to penetrate the basal lamina, neither* towards the epidermis nor towards the mucosa. None of the sections contained structures which could be identified as elastic fibres.

In all the studied drums a lamina propria was found also in the pars flaccida (Fig 5) It was less marked than in the pars tensa with a minor quantity of collagen fibres coursing into different directions.

MUCOSAL EPITHELIUM

Pars Flaccida

As already mentioned, the tympanic membrane was divided in the course of the dissection, into the pars flaccida, the area anterior to the manubrium and the area posterior to the manubrium In a few cases the latter two area were again divided into a central and a peripheral area

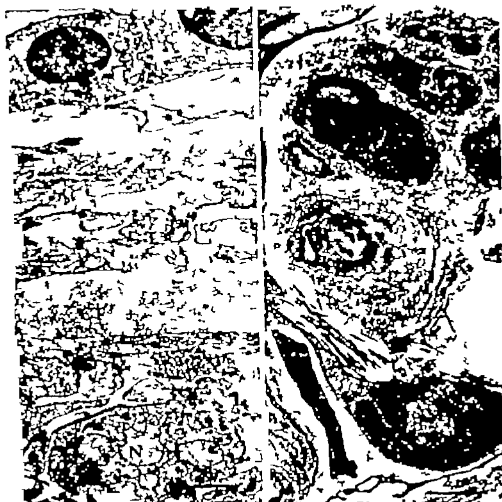


Fig 4 (*A*) Lamina propria and cuboidal cell of the mucous layer in the upper part of the picture. Nerve elements (N) and collagen fibrils (*CF*) are noted in the loose, connective tissue. (*B*) Nerve trunk in the tympanic membrane. Myelinated and unmyelinated nerves are noted as well as Schwann cell (*S*) with boundary layer. Abundant collagen fibers can be observed between the nerve fibers.

The epithelium at the site of the pars flaccida was built up, in all the drums, of simple squamous or cuboidal cells with microvilli, but without cilia or secretory granules (Fig 5) The cytoplasm housed a Golgi complex, granular reticulum, and a few mitochondria. The epithelium bordered on the underlying loose connective tissue by a basal lamina. Adjoining cells were connected, at the surface by a zonula occludens. The nuclei were ovoid and the nucleo–cytoplasmic ratio high The cell borders showed pronounced interdigitation.



Fig 5 Pars flaccida showi g impl squamo m cosal cell with microvilli nd der lyi g lamina propria.

Pars Tensa

The epithelium on the pars tensa varied in height, from low simple squamous or cuboidal (Fig 6) to pseudostratified columnar epithelium (Fig 7) The nuclei were ovoid, frequently with numerous crypts. The cell bor ders were characterized by pronounced interdigitations, while in the lower epithelium they were smooth. There were no goblet cells or glands, but in several places a basal cell. On the surface of the simple squam u cells microvilli, but no cilia, were observed In places where the epithelium was cub idal or taller there were cilia but most pronounced in the c lumnar epithelium Regardless of the height of the epithelium the cilia did not seem to form a continuous layer but to alternate in patches, with microvilli These appearances were found anteriorly and posteriorly a well as centrally and peripherally on the drum

The cilia exhibited the usual structure consisting of nine pairs of outer filaments, two central filaments, and at the base a basal corpuscle

At the site of the cell junctions there were immediately beneath the sur face zonula occludens, zonula adherens, and macula adherens. Al ng the other cell borders scattered desmosomes were observed.



Fig. 6. Mucosal layer of the tympanic membrane behind the manubrium. The cells are simple squamous with microvilli but without cilia.

The cytoplasm housed numerous round or ovoid mitochondria, ample granular reticulum and ribosomes, and a Golgi complex. The non ciliated cells frequently contained many dark secretory granules. The epithelium was delimited from the underlying connective tissue by a basal lamina.

Tympanic Membrane from Patients with Chronic Otitis Media

These preparations, fixed in osmium tetroxide immediately after removal were included in the study in order to ascertain whether the cell components were preserved in our method in which fixation with glutaraldehyde took place from 55 min to 4 hours *post mortem*. This proved to be so. Neither nuclei mitochondria, Golgi complex, nor endoplasmic reticulum was better preserved in this preparation than in the others. The time limit appears to be around 3 hours, a preparation fixed 3 hours *post mortem* proving well preserved, while another one fixed 4 hours *post mortem* showed structural damage of the cell organelles (Fig. 8).

The epithelium on the drum from the patients with chronic otitis media was pseudostratified columnar with numerous goblet cells containing large quantities of mucigen granules and having a surface densely studded with cilia. No glands were observed (Fig. 9).



Fig 7 Pseudostratified columnar epithelium of the tympanic membrane in front of the manubrium. Several cilia and secretory granules (*SG*) are observed. Cell membranes show marked interdigitation. Arrows indicate zonula occludens, zonula adherens and macula adherens. Desmosomes (*D*) and abundant granular reticulum (*GR*) and mitochondria (*M*) are noted.

DISCUSSION

The epidermis of the tympanic membrane showed no difference from normal cutaneous epidermis (Zelickson, 1967) also not in the pars flaccida. Melanin particles were not observed. In Lim's study (1968) of the ultrastructure of the tympanic membrane of the guinea pig, cat, and squirrel monkey the latter animal exhibited a terminal nerve which appeared to be situated between the squamous cells in the epidermis. No such finding was made in the present human material.

The lamina propria was found to consist of two layers of collagen fibrils as well as of loose connective tissue mainly submucous. This loose connective tissue housed numerous major and minor vessels as well as myelinated and unmyelinated nerve fibres. No nerve terminals were seen. Nowhere did



Fig. 8. Micrograph of the mucosal layer from specimen fixated 4 hours *post mortem* showing structural damage of autolysis. Empty areas are seen along the basal lamina. Broken membranes (arrows), swollen and disorganized mitochondria and dark cells are observed.

vessels or nerves penetrate the basal lamina, neither towards the epidermis nor towards the mucosa. No section showed structures that could be interpreted as elastic fibrils. However their existence cannot be ruled out, as they are difficult to identify electron microscopically on the basis of morphology alone (Zelickson, 1967).

In the course of time opinions have differed as to whether the epithelial cover of the tympanic cavity was simple flat, endothelial or taller columnar epithelium resembling that of the respiratory tract, but it seems to be agreed that the epithelium on the inside of the tympanic membrane is flat simple squamous or low cuboidal. Bueh (1967) found columnar epithelium in the tympanic cavity to merge into one layer of squamous cells at the site of the tympanic membrane. Sade (1966), studying temporal bones from 80 patients, found cilia to be widespread in the tympanic cavity but never present on the drum.

So far there have been only two papers on the ultrastructure of the epithelium in the auditory tube, tympanic cavity and on the tympanic membrane (Lim *et al.* 1967, Lim 1968). The temporal bones were derived from guinea pig, cat, and squirrel monkey.



Fig 9 Mucosal layer f th tympa ic membrane from pati nt with chronic ot ti media. T ll columna ciliated cell d goblet cell with immature (*MG*) nd mat re mucigen *gra ula re observed.*

Lim found the epithelium of the tympanic cavity in the guinea pig to be *comprised predominantly of simple squamous epithelial cells,* but occasionally small patches were observed with ciliated, cuboidal cells containing large dark granules. In the auditory tube there were tall pseud stratified,

ciliated columnar cells interspersed with goblet cells. The mucous layer on the drum proved to consist of simple squamous epithelial cells without cilia. Only along the edge of the drum were there cuboidal cells with or without cilia.

In man the appearances seem to be different, as the present material showed tall, at times even pseudostratified columnar epithelial cells with cilia and at times secretory granules in several sites of the tympanic membrane, *int. al.*, immediately anterior to the manubrium and on the posterior half. This applies only to the pars tensa, as the epithelium of the pars flaccida consisted in all cases of simple squamous or cuboidal cells without cilia or secretory granules.

Another interesting finding is that under pathological circumstances this epithelium may be converted into a more actively secreting epithelium with goblet cells. It is uncertain whether the goblet cells arise by conversion of ciliated cells, as suggested by Spoendlin (1959) or by conversion of basal cells, as suggested by Rhodin (1959)—both of whom based their conclusions upon the upper respiratory epithelium.

Out of regard to future studies it is important to know that up to 3 hours may elapse *post mortem* before fixation of the tympanic membrane, without essential changes taking place in cellular ultrastructure.

ZUSAMMENFASSUNG

Eine elektronenmikroskopische Untersuchung der normalen Mittelohrschleimhaut beim Mensch ist angefangen worden, um durch in besseres Wissen von der Ultrastruktur des Mitt lohres ein besseres Verstandni von den Mitt lohrkrank heiten u bekommen Di Präparate wurden von 55 Minuten bis 4 Stunden nach dem Tod fi iert. Ei postmortales I tervall über 3 Stund n hat ut lytisch Ver änderungen verursacht. Das Trommelf ll bestand aus 3 Schichten in äussere Epidermisschicht, deren Ultrastruktur wi die der normalen Haut war die Lamina propria mit lockerem Bi degeweb, Blutgefässen und Nervenfasern und zwei Kollagenfibrillenschichten (uch in der Pars flaccida) u d schliesslich di Lamina mucosa, die in der Pars flaccida us ei em einfachen Plattenepithel mit Mikrovilli bestand. Überall auf der Pars tensa bestand die Mucosa aus Zellen von wechselnder Höhe oft wurd ein geschichtetes ziliäres Zyli derepith l mit dunk len Sekreti nsgranula gefunden. Becherzellen wurden i der Schleimhaut des normalen Trommelf lls ni ht g funden. In einem Fall von chronischer otitis media wurden aber manche Becherz llen gefunden. Di normale Schl imhaut des Trommelf lls hat nur in schwache Sekreti n Unter path logischen Umständen kö en Becherz llen und ei lebhaft Sekretion uftreten.

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EUSTACHIAN TUBE FUNCTION IN PATIENTS WITH EAR DRUM PERFORATIONS FOLLOWING CHRONIC OTITIS MEDIA

Results of a Simplified Testing Procedure by Deflation and Aspiration Methods

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During the last decade methods allowing adequate assessment of the Eustachian tube function have been developed. The author has simplified these new methods, the "deflation and aspiration methods" for clinical use. The equipment and the performance have been described and the reproducibility has been analyzed. 217 ears with central ear drum perforations following chronic otitis media have been tested and the results discussed. The residual pressure levels seem to be the most reliable way of grading the deflation and aspiration methods. Complete equalization in the range of 0 to 20 mm H_2O was achieved in 35% of the ears with positive pressure and in 23% of the ears with negative pressure. The simplified testing procedure presented in this report is an easy, rapid and reliable way to assess tubal function and provide important information.

As has been stressed repeatedly by leading otologists (Brockman, 1901, Wullstein, 1963, Zöllner 1963, Farrior 1965, Schuknecht & Kerr 1967 etc.) Eustachian tube dysfunction is certainly one of the most important factors in the etiology and course of infectious middle ear diseases. Parallel with the development of modern reconstructive middle ear surgery, the need for methods allowing adequate assessment of tubal function has become evident. During the last decade such methods have also been developed (Holborow 1962, Flisberg *et al.* 1963, Miller 1965, Flisberg, 1966, Siedentop *et al.*, 1968). The principle of these so-called deflation and aspiration methods, is to create well defined positive and negative middle ear pressure levels and to register the way and degree of equalization upon swallowing.

In spite of this, the deflation and aspiration methods do not seem to have been generally accepted for clinical use. The main reason for this is that we still only have limited documentation of the prognostic value of these methods (Siedentop *et al.* 1968, Holmquist, 1968).

Since 1964 the author has used the deflation and aspiration methods in

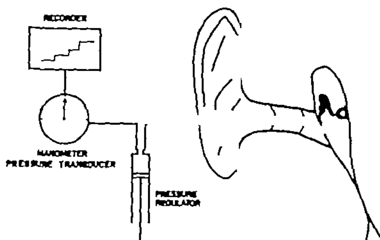


Fig 1 Diagram f apparat s for testing East chian tube f ction.

the tests of tubal function of more than 1000 ears. The aim of this paper is to describe the technique used, to analyze the reproducibility of the methods and to present test results of 217 ears selected for tympanoplasty

METHODS

The equipment used in this investigation is the same as that introduced by Flisberg *et al* in 1963

Equipment

The testing situation is shown in Fig 1 The patient is in a sitting position. A plastic foam cuff with a metal tube (inner diameter 2 mm) is placed in the ear canal The foam cuff is used instead of the inflatable balloon cuff used by Flisberg *et al* (1963) It is necessary to make sure that the connection to the ear canal is air tight and well permit the artificial pressure levels. *A pressure regulator and a pressure transducer with a manometer* (EMT 33 and EMT 31 Elema Schönander AB, Stockholm Sweden) are connected to the metal tube. The connection consists of plastic tubing with an inner diameter of 3 mm and a length of 250 mm. The short connection will give almost the same pressure in the ear as in the pressure manometer and the small differences are of no practical importance The volume of the system outside the cuff is constant With the aid of the pressure regulator a simple syringe the desired positive and negative initial pressure levels can be arranged The recordings are made with an ink writer (Mingograph 34 *Elema-Schönander AB Stockholm Sweden*)

Performance

Whenever possible a standardized testing procedure was used By means of the syringe a positive pressure level of +200 mm H_2O above atm pheric

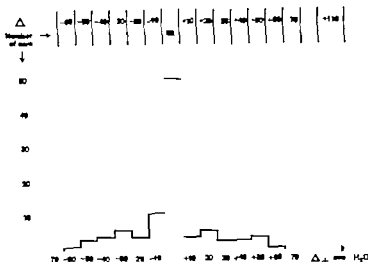


Fig 2. Differences between two examinations of residual pressure in mm H_2O with deflation method in 101 ears. $\Delta_+ = Y - Y$ where Y is the first examination and Y is the second examination. In the range of −15.0 to +15.0 is 65% of the observations. In the range of −45.0 to +45.0 is 91% of the observations. Average value of Δ_+ is −3.52. Standard deviation for Δ_+ is 30.6.

pressure was first created. This was done in 2 or 3 sec. (Sometimes this positive pressure level was not reached. The tube opened spontaneously without swallowing as a result of the force of the pressure.) When a constant pressure was reached the patient was told to swallow repeatedly. Often some water was offered to the patient to facilitate swallowing. The patient was also told to press his teeth together to avoid movements of the cuff. The pressure changes in the middle ear were recorded continuously. This procedure was then repeated by using 200 mm H_2O as the initial pressure level and the pressure changes during swallowing again recorded.

The recordings have been analyzed with regard to the residual pressure levels where further swallowings did not produce any pressure changes.

Statistical notes

Since the reproducibility of the deflation and aspiration methods has previously not been well documented, it was of interest to analyze the experimental error. For this purpose recordings were duplicated in 101 ears by the deflation method and in 85 ears by the aspiration method. The ages of the patients were between 14 and 70 years. The patients were of both sexes. The perforations were dry; the retesting was made in 24 hours. Control was made that no upper respiratory disorders were present during the course of the study.

I collaboration with Ass. Göran Thorén, Chalmers University of Technology, Göteborg.

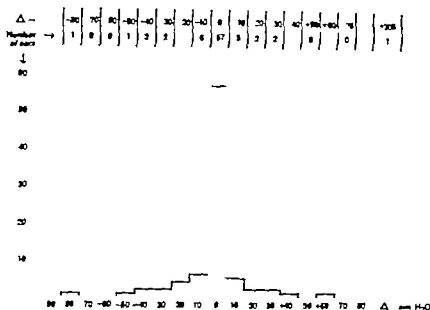


Fig 3 Differences between tw examinati ns f residual pressure i mm H_2O with spirati n method in 63 ears. $\Delta = X - X$ where X i the first examination nd X i the second examination. In the range of −15.0 t +15.0 i 80% f the bservatio s, i the ra ge f −45.0 t +45.0 i 83 . f the bservati ns. A erage v lue for $\Delta = -0.31$ Standard devi tion f $\Delta = 17.1$

The results are presented in Fig. 2 for the deflation method and in Fig 3 for the aspiration method In Fig 2 we observe that in the range of −15 to +15 mm H_2O (= 15% of the total range) we have 65% of the observations with deflation method In Fig 3 we observe that in the range of −1 to +15 mm H_2O (= 15% of the total range) we have 80% of the observations with aspiration method The calculation of the standard deviation gave a figure of 21 6 with the deflation method and 12 1 with the aspiration method. *From a medical point of view these values were considered satisfactory*

Comments

Flisberg and even Miller use a whole series of initial pressure levels in their test proceedings The procedure described above differs from these in that only two initial pressure levels, +200 and −200 mm H O h e been used This measuring procedure is less time-consuming as a rule it took *less than 5 min This has also meant that the risk of eventual changes in the* muc us membrane of the middle ear and the Eustachian tube from artificial pressure has been reduced. These changes increase with time and rising pressure levels and it is therefore an advantage for the test proceedings to go quickly The registrations are in this way easier to survey an l comparisons between various registrations are carried out more quickly

The balloon in the ear canal, recommended by Flisberg has been ex changed for a plastic foam cuff which, *according to the auth r , h es a* safer and easier adaption to the lumen of the ear canal

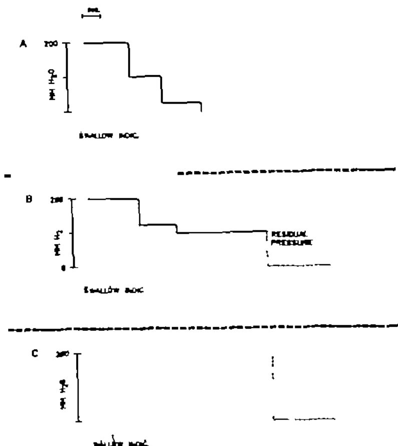


Fig 4 Patterns of recordings with deflation method. (A) Equalization of the intratympanic positive pressure. At each swallow there are pressure drops until zero is reached. (B) Partial equalization. A residual pressure is recorded. (C) No pressure changes are produced by swallowing.

MATERIAL

Two hundred patients (217 ears) with persistent central ear drum perforations following chronic otitis media have been tested. The ages of the patients were between 8 and 72 years in 95 men and 105 women. All ears were dry and no signs of infection were present at the time of testing. The measurements were included in a pre-operative routine testing battery used in patients seeking tympanoplasty. It has been avoided to make measurements in connection with allergic manifestations or upper respiratory infections, when tubal function may change.

RESULTS

Deflation method

Examples of tracings from three different patients tested by the deflation method are shown in Fig 4.

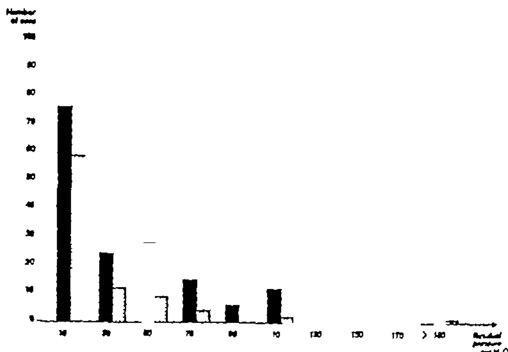


Fig 5. Histogram showi g results with deflatio method. ■ Indicates numbe of ears grouped ccording to the residual pressure with deflation method. □ Indicates number f rs in the group ble t hang th negative pressure more than 100 mm H_2O

The results of the total series are illustrated in Fig 5 where the ears have been divided into different groups according to the residual pressure level. In each group the number of ears, which were able to change the negative pressure more than 100 mm H_2O are also shown. With increasing residual pressure by deflation method a declining percentage of ears with ability to change negative pressure more than 100 mm H_2O is found

The positive pressure was normalized in 35% (70 ears) of the ears. In this group a small residual pressure up to 20 mm H_2O was rec rded The positive pressure was only partially equalized in 43% of the ear (84 ears) Varying residual pressure levels were recorded The positive pressure was not reduced or reduced less than 20 mm H_2O in 22% (4 ears f the ears.

Aspiration method

Examples of tracings from three different cases by aspiration method are shown in Fig 6

The results of the total series are illustrated in Fig 7 where th ars have been divided into different groups according to the residual re ure level. In each group even the number of ears, which were able to d crease the positive pressure more than 100 mm H_2O are shown All ears, except 5, which could change the negative pressure could also reduce the p itive pressure more han 100 mm H_2O

The negative pressure was equalized in 22 of the ears (49 ears In this

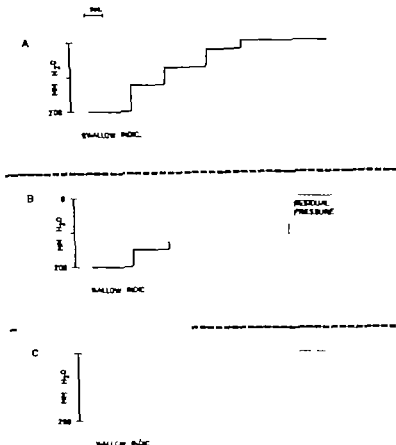


Fig 6 Patterns of recording with aspiration method. (A) Equalization of the intra tympanic negative pressure At each swallow there are pressure changes until zero is reached. (B) Partial equalization. A residual pressure is recorded. (C) No pressure changes are produced by swallowing.

group a small residual pressure of less than 20 mm H_2O was recorded The negative pressure was only partially equalized in 26% of the ears (56 ears) Varying residual pressure levels were recorded. The negative pressure was not changed or changed less than 20 mm H_2O in 52% of the ears (112 ears)

DISCUSSION

As stated by many authors (Thomsen, 1958 Ingelstedt *et al.*, 1963) classical tubal function tests give only rough or sometimes even incorrect estimates of the ventilating function of the Eustachian tube. By the new methods introduced—deflation and aspiration methods—we are able to test the tube in a more physiological way by determining the ability to equalize artificial, well defined positive and negative middle ear pressure

It is obvious that increased positive middle ear pressure facilitates

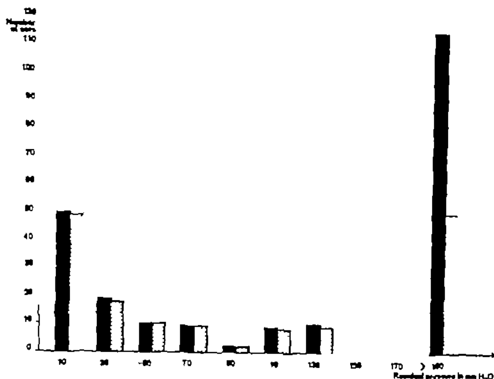


Fig 7 Hist gram showing results with aspirati n method ■ Indicates number f ears grouped according t the residual pressure with spiration method □ Indicates number f ears in the group ble t cha ge the po iti pressure more tha 100 mm H_2O

opening of the tube upon swallowing Even if the situation is more complex the same holds true for negative pressure up to −200 mm H_2O At higher negative middle ear pressure levels there are more complicated conditions which influence equalization as i.e. the locking phenomena

One question which the author has investigated, is if there are any differences between the testing results in rapid change compared to stepwise change of the negative artificial pressure Fig 8 shows one case tested with stepwise increasing negative pressure This shows that not until artificial pressure at −150 mm H_2O is the patient able to change the pressure upon swallowing and a residual pressure is recorded (−60 mm H_2O) This case has also been tested with rapid change of the middle ear pressure to −200 mm H_2O and swallowing, according to the testing procedure introduced The same residual pressure level was recorded Many ears have been tested by both procedures. The results are compared and found to be in agreement Thus the method introduced seems to be well adapted for determination of residual pressure levels

In order to quantify the testing results many parameters can be used, as for example the least positive or negative pressure difference across the tube giving air passage upon swallowing, number and size of pressure drops at swallowing and residual pressure levels Flisberg (1966) has looked at these parameters in many ways, but he does not indicate which one accord

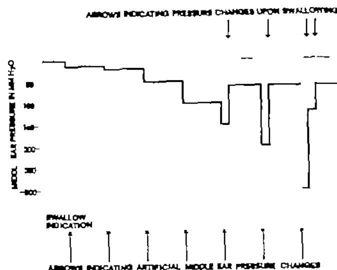


Fig 5 Pressure chang recording from ar case The rtificial middle ea pressure ha been cha ged stepwise t −300 mm H_2O.

ing to his opinion is the most significant Miller (1965) has adopted the negative residual middle ear pressure as the simplest and most accurate parameters are dependent upon this.

From a physiological point of view the least positive and negative pressure difference across the tube giving air passage upon swallowing seem to be the most sensitive parameters for estimation of the tubal function However measurement of these parameters is too complicated and timeconsuming to be used as a routine in clinical tests An acceptable compromise therefore seems to be the use of the residual pressure levels, which are reached after repeated swallowings with initial middle ear pressure levels of +200 and −200 mm H_2O The good reproducibility of the measurements also shows that the residual pressure levels are adequate parameters of the tubal function.

The simplified way of measuring used in this investigation puts the question are the results in accordance with those published earlier on the tubal function in ears with chronic otitis media, judged by deflation and aspiration methods (Miller 1965 Flisberg, 1966 Siedentop, 1968) Unfortunately differences existing between these investigations regarding selection of material as well as documentation, makes a thorough comparison impossible However regarding the residual pressure level less than −50 mm H_2O by the aspiration method, the results can be compared and are well in accordance with each other

The 217 ears in this investigation are selected for tympanoplasty from a larger material of chronic otitis media. Even between these selected ears there exist great variations in tubal function. 22% of the ears could not at all reduce +200 mm H O applied in the middle ear while as many as 52%

could not change a negative pressure. Complete equalization was achieved in 35% with positive and 23% with negative pressure.

It would be premature to establish groups in a material like this in terms of normal and different kinds of pathological tubal function. The only method of classification in this respect is to correlate between tubal function test and the clinical course in operated as well as unoperated ears over a period of many years. However the results of a 1 year follow up study show a good correlation between pre-operative tubal function and healing after myringoplasty (Holmquist 1968).

The simplified testing procedure presented in this investigation is an easy and rapid way of assessing tubal function. It gives important and reliable information and should be included in the routine testing battery like stereomicroscopic study, X-ray investigation, audiometry etc.

ZUSAMMENFASSUNG

In dem letzen Jahrzehnt sind neue Methoden entwickelt worden die erlauben die genaue Funktion der Tuba Eustachii festzustellen. Der Verfasser hat diese „Deflations" und „Aspirations" Methoden für klinischen Gebrauch vereinfacht. Die zur Durchführung dieser Methode benötigte Apparatur und deren Anwendung ist beschrieben und die Reproduktibilitet der Methode untersucht worden. 217 Ohren mit centralen Trommelfellperforationen als Folge kronischer Otiten sind nach dieser Methode untersucht und die Resultate diskutiert worden.

Die Untersuchungen zeigten, dass die Verwendung des Restdrucknivеaus der sicherste Weg zur Gradierung der Deflations- und Aspirations-Methoden ist. Vollkommenen Druckausgleich, zwischen 0 und 20 mm H_2O erreichte man bei 35% der Ohren mit Überdruck und bei 23% mit Unterdruck. Die hier beschriebene vereinfachte Untersuchungsmethode ist ein einfaches, schnelles und reproduktiebles Verfahren, auf dem die Untersuchung der Tuba Eustachii mit sehr gutem Resultat durchgeführt werden kann.

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AUDITORY FUNCTIONS IN RAISED INTRACRANIAL PRESSURE

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A study of auditory functions was made in 25 cases of raised intracranial tension. It was noted that an average loss of 30 dB could occur due to raised intracranial pressure and that this improves after the return of normal pressure in the cranium, as seen in 16 cases submitted to surgery Audiometric examination has been found to be a valuable diagnostic investigation in cases of raised intracranial tension

Whilst dealing with rather advanced cases of intracranial space-occupying lesions our neurosurgical colleagues were struck by a high incidence of symptoms referable to VIII nerve even when the cause or raised intra cranial pressure was away from this nerve We therefore instituted a col laborative study of the effects of raised intracranial pressure on cochlear and vestibular functions. During the last year we have studied 60 patients but for the purpose of this report we have selected first 25 cases to illustrate that the clinical impression finds significant confirmation from toneurological investigations. The auditory function tests revealed a significant impairment although routine tests for vestibular functions (cal ric test, rotation test, Fukuda s writing test and Fukuda s stepping test) were not much altered This report, therefore is restricted to the study of udit ry functions only

MATERIAL AND METHODS

The present report is based on 25 cases of raised intracranial pressure admitted to the neurology and neurosurgery wards of the All India In titute of Medical Sciences, New Delhi The criteria for selection of such patients was the presence of clinical or radiological evidence of raised intr ranial pressure Besides the usual symptoms of raised pressure e.g h lache vomiting and visual changes, every patient included in the tudy had p pillo-edema

The radiological signs which were taken into considerati n were para-

Presented t the Scientifi sessio f the l conference f the \ soci ti f Oto-laryngol gist f India, h ld t Poona in J uary 1967 Thi work w support d by grant from the Council of Scientific nd Industrial Rese rch of India.

Table 1 *Analysis of 25 cases of intracranial pressure*

Site of lesion	Cases Pre-operative	Operated	Expired	Post perative follow-up
Frontal lobe tumour	10	8	0	6
Parietal lobe t mour	6	5	0	5
Temporal lobe tumour	5	4	0	3
Pituitary tumour	1	1	1	0
Hydrocephalus	3	3	1	2
Total	25	21	2	16

tion of sutures, decalcification and erosion of dorsum sella or enlargement of the pituitary fossa, enlargement of emissary foramina in the occipital bone and increased convolutional markings.

The cause of raised intracranial pressure was established by contrast radiography e.g angiography and air or myodil ventriculography In 21 cases this was confirmed by surgery

Clinical E.N T examination of all the patients was done before doing audiometric studies. Examination of auditory functions included the follow ing tests (*a*) Pure-tone audiometry (The average hearing loss in these cases has been calculated as the mean of hearing loss at frequency of 500 1000 2000, 4000 cycles/sec.) (*b*) Recruitment test *ad modum* Lüscher & Zwislocki (1948) (*c*) Tone-decay test *ad modum* Carhart (1957)

Out of the 25 cases, 21 cases were submitted to surgery and tests were repeated in 16 cases during the post-operative period (Table 1)

RESULTS

Twenty five cases of raised intracranial pressure were studied, thus mak ing 54 ears in all Hearing disorder was found in 44 ears and normal hearing was f und only in six ears whereas only two patients had complained of impaired hearing during interrogation. Slight (15–30 dB) hearing loss was present in 60% of cases, moderate (31–60 dB) in 26% and severe (above 00 dB) in 2% of cases. The type of hearing loss which was encountered in these cases was found to be conductive type in 33% of cases, perceptive type in 52% and mixed type in 15% of cases. Various other factors producing these types of hearing losses could be excluded on the basis of history (e.g. history of taking ototoxic drugs, exanthemata etc.) and clinical examination (T.M was normal in the cases studied and Eustachian tube function were normal as tested by Valsalva s manoeuvre and Eu ta hian catheterization) All those cases who were considered to be mentally un sound (as tested by neurological examination) and non-cooperative for the

Table 2 *Audiometric changes in cases of raised intracranial pressure due to different sites of lesion*

Site of lesion	No of cases	Laterality of ear	Pure tone (hearing loss in dB) (0–15)	(16–30)	(31–60)	(61–90)	(above 90 dB)	Recruitment −	±	+	++	Tone decay −	±	+
Frontal lobe	10	Homo-lateral	2	3	4	1	0	8	1	0	1	9	1	0
		Contra-lateral	1	7	2	0	0	7	2	0	1	8	0	2
Parietal lobe	6	Homo-lateral	1	3	2	0	0	5	0	1	0	6	0	0
		Contra-lateral	1	3	2	0	0	5	0	1	0	6	0	0
Temporal lobe	5	Homo-lateral	1	2	2	0	0	2	2	1	0	5	0	0
		Contra-lateral	0	4	1	0	0	4	0	1	0	5	0	0
Pituitary tumour	1	Right	0	1	0	0	0	1	0	0	0	1	0	0
		Left	0	1	0	0	0	1	0	0	0	1	0	0
Hydrocephalus	3	Right	0	3	0	0	0	1	2	0	0	3	0	0
		Left	0	3	0	0	0	1	2	0	0	3	0	0
Total	25		6	30	13	1	0	35	9	1	2	46	1	3
Percentage			12	60	26	2	0	70	18	2	4	92	2	6

Note Normal hearing (0–15 dB loss) calculated as = ¼ (500 + 1000 + 2000 + 4000); mild hearing loss (16–30 dB loss) calculated as = ¼ (500 + 1000 + 2000 + 4000); moderate hearing loss (31–60 dB loss), calculated as = ¼ (500 + 1000 + 2000 + 4000), profound hearing loss (61–90 dB loss), calculated as = ¼ (500 + 1000 + 2000 + 4000); severe hearing loss (above 90 dB loss), calculated as ¼ (500 + 1000 + 2000 + 4000).

Recruitment. − denotes 1 to 6, ± 0.75, + 0.5, ++ 0.25 dB difference limen reading.

Tone decay − means up to 1-step rise ± up to 2-step rise + more than 2-step rise

tests were excluded from the study However such cases were very few The results of auditory function tests are shown in Table 2.

Twenty-one patients were submitted to surgery in order to relieve the raised intracranial pressure, and study of auditory functions could be done in 16 cases during the post-operative period (two cases having expired and three were not co-operative for study during post-operative period) Improvement in hearing was found in a significant number of cases as is shown in Table 3

In order to illustrate the results a few case reports are given below

Case 1

M R 35 male was admitted on 14-5 1966 with complaints of headache and vomiting of 6 months duration, impairment of vision from both eyes

Table 3 *Comparison of auditory functions in pre-operative and post-operative period in percentage*

	Pure tone (hearing loss in dB)					Recruitment				Tone decay		
	Normal (0–15)	Slight (16–30)	Moderat (31–60)	Severe (61–90)	Profound (above 90)	−	±	+	++	−	±	+
Pre-operative (25 cases)	12	60	26	2	0	70	18	8	4	92	2	6
Post-operative (16 cases)	43	45	12	0	0	97	0	3	0	100	0	0

Symbol explained in T ble 2.

for the last 4 months, impairment of hearing and tinnitus right ear for 2 months. On general physical examination he was found to be a well-built man. On examination of nervous system he was found to have normal intelligence and was well orientated in time and place. His speech and memory were normal He had bilateral papilloedema (3 diopters) with haemorrhage and concentric constriction of visual fields. Paresis of right trigeminal nerve especially in the distribution of 1st division, bilateral paralysis of VI nerve and supranuclear type paralysis of VII nerve on right side. Cerebellar signs were absent. On examination of ear nose and throat no abnormality was detected. Routine laboratory investigations were not contributory to any cause of the disease Skull X ray proved the presence of raised intracranial pressure Ventriculogram and angiogram revealed a cystic space-occupying lesion in the right fronto-parietal region. Pure tone audiogram showed profound hearing loss on right side and mixed deafness on left side tone decay was present on left side. Recruitment and tone decay tests could not be done on right side due to the presence of profound hearing loss. Exploration was done on 6.4.66 and meningio-epithelioma was excised from right fronto-parietal region. Cochleo-vestibular function tests were repeated on 18.4.66, 3.6.66 and 22.9.66 Results of hearing tests are shown in Fig. 1 Subjectively patient also had better hearing and better vision.

Case 2

N S., 29 male, presented on 20.5.66 with the complaints of continuos headache for the last 3 years and impairment of vision for 2 years. The headache had been continuous, diffuse mainly localised to left temporal regi n and not accompanied by nausea or vomiting. He was also having progressive impairment of vision from both eyes, more on left than right and used to get diplopia on looking towards left. Three months prior to admission the patient had complete loss of vision in the left eye and 15 days prior to admissi n he began to feel a weakness of the left half of body

Table 2 *Audiometric changes in cases of raised intracranial pressure due to different sites of lesion*

Site of lesion	No. of cases	Laterality of ear	Pure tone (hearing loss in dB) (0–15)	(16–30)	(31–60)	(61–90)	(above 90 dB)	Recruitment –	±	+	++	Tone decay –	±	+
Frontal lobe	10	Homolateral	2	3	4	1	0	8	1	0	1	9	1	0
		Contralateral	1	7	2	0	0	7	2	0	1	8	0	2
Parietal lobe	6	Homolateral	1	3	2	0	0	5	0	1	0	6	0	0
		Contralateral	1	3	2	0	0	5	0	1	0	6	0	0
Temporal lobe	5	Homolateral	1	2	2	0	0	2	2	1	0	5	0	0
		Contralateral	0	4	1	0	0	4	0	1	0	5	0	0
Pituitary tumour	1	Right	0	1	0	0	0	1	0	0	0	1	0	0
		Left	0	1	0	0	0	1	0	0	0	1	0	0
Hydrocephalus	3	Right	0	3	0	0	0	1	2	0	0	3	0	0
		Left	0	3	0	0	0	1	2	0	0	3	0	0
Total	25		6	30	13	1	0	35	9	4	2	48	1	3
Percentage			12	60	26	2	0	70	18	8	[illegible]	92	2	6

Note. *Normal hearing (0–15 dB loss) calculated as* = ¼ (500 1000 2000 4000), *mild hearing* loss (16–30 dB loss), calculated as = ¼ (500 + 1000 + 2000 + 4000); moderate hearing loss (31–60 dB loss), calculated as = ¼ (500 + 1000 + 2000 + 4000); profound hearing loss (61–90 dB loss), *calculated as* = ¼ *(500 + 1000 + 2000 + 4000); severe hearing loss (above 90 dB loss), calculated* as = ¼ (500 + 1000 + 2000 + 4000).

Recruitment – denotes 1 to 6, ± 0.75, + 0.5, + + 0.25 dB difference limen reading.

Tone decay – *means up to 1-step rise ± up to 2 step rise more than step rise*

tests were excluded from the study. However, such cases were very few. The results of auditory function tests are shown in Table 2.

Twenty-one patients were submitted to surgery in order to relieve the raised intracranial pressure, and study of auditory functions could be done in 10 cases during the post-operative period (two cases having expired and *three were not co-operative for study during post-operative period).* Improvement in hearing was found in a significant number of cases as is shown in Table 3.

In order to illustrate the results a few case reports are given below.

Case 1

M. R. 35 male was admitted on 14.5.1966 with complaint of headache and vomiting of 9 months duration, impairment of vision from both eyes

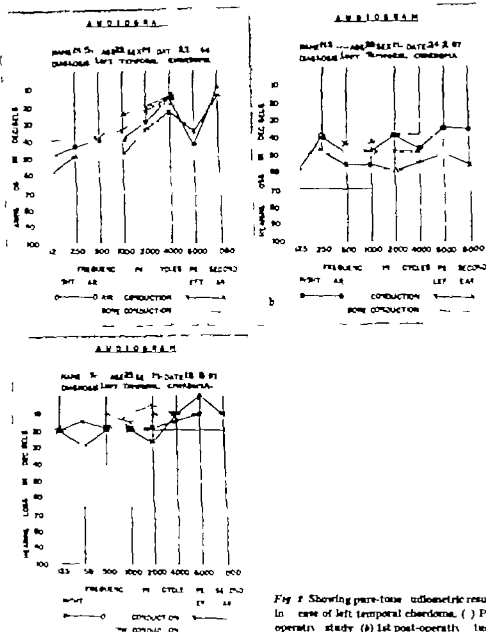
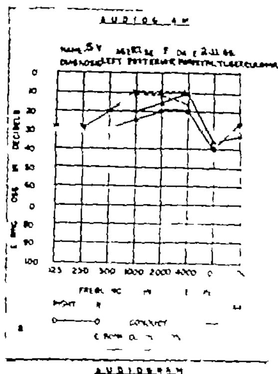
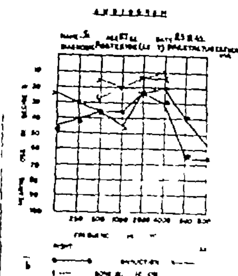


Fig 2 Showing pure-tone audiometric results in case of left temporal chordoma. ( ) Pre-operative study (*b*) 1st post-operative study ( ) 2nd post-operative study

revealed evidence of raised intracranial pressure Audiometric tests revealed bilateral moderate sensorineural type of hearing loss without the presence of recruitment or tone decay The patient was submitted for operation and chordoma from left temporal region of brain was excised on 3.6.66. Auditory and vestibular function tests were repeated in the post-operative period Results of auditory function tests are shown in Fig. 2.





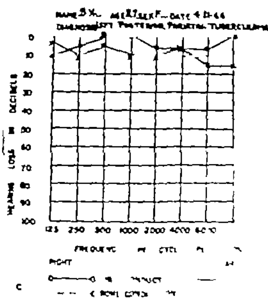


Fig 4. Showing pure-tone audiometric result in case f left posterior parietal tuberculoma. ( ) Pre-operativ study (*b*) 1st post operative study ( ) 2nd post-ope t study

Case 3

S. V., 2/ female was admitted on 22.10.65 with the chief complaint f headache and vomiting and impairment of vision in both eyes f r the la t 3 months. Headache was localised in the occipital region On x mination she was fully conscious and well orientated her peech, gait and mory were normal she had bilateral papilloedema but vision was 6 6 Th e was

no neurological deficit to pinpoint the site of lesion. Examination of ear nose and throat was non-contributory Audiometric test revealed slight hearing loss, conductive type, in both ears, without recruitment or tone decay Ventriculography revealed a mass in left parieto-temporal region. A tuberculoma from left posterior parietal region was excised on 10 11 65 Auditory and vestibular functions were repeated on 23.11 65 and 4.11.66 Results of auditory functions (Fig. 3) show improvement in functions in post-operative period

DISCUSSION

The problem of hearing loss in cases of raised intracranial pressure has rarely been studied in the past. Genkin & Ebich (1958) found reduction in hearing sensitivity in cases of raised intracranial pressure, using tuning forks for their study Allen & Habibi (1962) noticed a reduction in amplitude of cochlear microphonics after increasing cerebrospinal fluid pressure. Klockhoff *et al* (1964 and 1966) also found that an increase in intracranial pressure is transmitted to the inner-ear fluids resulting in a change of acoustic impedance of the middle ear They confirmed that this change in acoustic impedance of the middle ear is due to the mechanical hood of inner ear fluids on stapedial foot plate. Brunetti & Menzio (1951) found that variations in cerebrospinal fluid pressure could be transmitted to the fluid system of the labyrinth via cochlear aqueduct and this causes variations in stimulus threshold of cochlear receptors.

The present study of auditory functions in cases of raised intracranial pressure revealed that 85% of the cases of raised intracranial pressure due to a variety of lesions in the supratentorial region were associated with a significant degree of hearing impairment. The average hearing loss was 30 dB. This finding is identical with that of Hattori (1961) who found the same degree of hearing loss in cases of brain tumour The nature and site of the intracranial lesion were so variable that it appears reasonable to ascribe the changes to be due to the effect of raised intracranial pressure which was a constant finding in all these cases. Factors like previous ear disease and use of ototoxic drugs were ruled out on the basis of the results of clinical and radiological investigations as well as of surgery It is reasonable to believe that the hearing loss was due to raised intracranial pressure because the hearing functions improved after surgical removal of the etiological facto responsible for raised intracranial pressure.

Theoretically speaking, hearing defects could result from direct or indirect involvement of either the conductive mechanism or the perceptive mechanism, or both. Before starting this study we expected that raised intracranial pressure—should it produce alteration in hearing—would do so by its effect on the internal ear due to transmission of pressure to the perilymph via the cochlear aqueduct which may also directly or indirectly affect the endolymphatic system. The latter may be due to alteration around the endolymphatic sac or secondary to the perilymphatic changes. We therefore

expected to find our results identical to hydrops of the inner ear. The results of our study suggest, however, that there is more than one mechanism at play which is responsible for conductive loss in 33 %, perceptive loss in 52 % and mixed type of hearing loss in 15 % of the cases.

The hearing loss in these cases is unrelated to the site or nature of the lesion responsible for raised pressure though it is probably related to the severity of raised intracranial pressure. The improvement in hearing following relief of raised intracranial pressure strongly suggests that the hearing loss observed in these cases was due to this condition. It is felt that in those cases where hearing did not improve, permanent damage had occurred, just as optic atrophy occurs in cases of prolonged papilloedema.

ZUSAMMENFASSUNG

Es wird über die auditorischen Funktionen bei 25 Fällen von erhöhtem intrakraniellem Druck berichtet. Es wurde beobachtet, dass ein auf erhöhten intrakraniellen Druck zurückzuführender durchschnittlicher Verlust von 30 dB vorkommen konnte und dass sich dies verbesserte nach Rückkehr auf Normaldruck im Kranium, wie aus 10 operierten Fällen zu ersehen ist. Die audiometrische Untersuchung wird bei Fällen von erhöhtem intrakraniellem Druck als eine wertvolle Untersuchung empfunden.

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TEMPERATURMESSUNGEN AM MENSCHLICHEN BOGENGANG NACH THERMISCHER REIZUNG

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Außer einer Untersuchung an nur 2 Patienten liegen Temperaturmessungen am horizontalen Bogengang nach thermischer Reizung am Lebenden nicht vor. Bei 11 Ohroperationen wurde durch den Gehörgang (Radikaloperationen, Tympanoplastiken) oder retroaurikulär (Antrotomien) eine elektrische Meßsonde am Bogengangsmassiv angelegt und insgesamt 57 Temperaturverläufe nach thermischer Reizung (Füllung des Gehörganges mit 3 ml Wasser, Einwirkungszeit 20 sec) registriert.

25 Messungen mit 20°C-Reiz erbrachten im Durchschnitt eine Abkühlung von 0,8°C nach 40 sec, 15 Messungen mit 44°C-Reiz eine Erwärmung um 0,21 C nach 40 sec und 17 Messungen mit 0 C-Reiz eine Abkühlung von 1,2 C nach 70 sec. In allen Fällen anfängliche schnelle Temperaturveränderung bis zum Maximum mit anschließender langsamer Rückkehr zur Ausgangslage (Verhältnis 1:6). Daraus ergeben sich Erholungszeiten von 5 min für 44 Spülung, 8 min für 20 Spülung und 10 min für 0 Spülung. Nystagmus trat wegen der Untersuchung im Liegen nur 7 mal bei Eiswasserspülung auf. Er zeigte keine direkte Abhängigkeit von der Temperaturveränderung am Bogengang. Die in den Tabellen getrennt aufgeführten Werte der Antrotomiegruppen besitzen auf Grund der physiologischeren Versuchsbedingungen eine bessere Aussagekraft.

H. Frenzel schreibt zur Wirkung des kalorischen Reizes auf das Vestibularorgan: „Bei der thermischen Prüfung wird durch Kaltspülung oder Heißspülung des Gehörganges ein zum Schädelinneren fortschreitendes Temperaturgefälle erzeugt. Es erreicht den horizontalen Bogengang in seinem glatten Schenkel nahe der Ampulle am frühesten und kühlt hier die Flüssigkeitsteilchen der Endolymphe ab oder erwärmt sie." Frenzel begründet seine Ansicht mit im Jahre 1925 durchgeführten Temperaturmessungen an Felsenbeinpräparaten, die aber wegen der fehlenden Durchblutung nicht ohne weiteres auf die Verhältnisse am Lebenden übertragen werden können. Messungen an Felsenbeinen in ähnlicher Art liegen von Dohlman und Meurman vor. Eine Abkühlung trat bei Spülung mit 100 ml Wasser um 0,3 C (Dohlman, 1925) und bei Spülung mit 10 ml Wasser um 0,13 C ein (Meurman, 1924).

Lediglich Schmaltz & Vögler kamen 1924 durch Messungen an antrotomierten Patienten zu verwertbaren Ergebnissen. Sie stellten eine Abkühlung am lateralen Bogengang von 0,36 C nach Spülung mit 10 ml Eiswasser fest. Da von den Autoren Untersuchungen an nur 2 Patienten vor

liegen haben wir die Frage des Temperaturgefälles am horizontalen Bogengang nach thermischer Reizung noch einmal aufgegriffen und Vestibularisprüfungen mit 0 C, 20 C und 44 C durchgeführt

METHODIK

Der Zugang zum lateralen Bogengang wurde bei geeigneten Ohroperationen (Radikaloperation, Tympanoplastik und Antrotomie) vorgenommen und die Temperatur Meßsonde am lateralen Bogengang angelegt Dabei kamen Patienten zur Auswahl bei denen keine oder nur geringe Ohrsekretion bestand, um möglichst physiologische Verhältnisse zu wahren.

Bei Radikaloperationen und Tympanoplastiken wurde nach Abschieben des häutigen Gehörgangsschlauches eine Antrumbohrung bis in die Nähe des lateralen Bogenganges durchgeführt Die Öffnung nahm die Sonde auf die sich durch Biegen und Anklemmen des Drahtes am Bogengang unter Sicht fixieren ließ Nach Zurückklappen des häutigen Gehörgangsschlauches Messung der Ausgangstemperatur Bei bestehender Perforation des Trommelfells mußte die offene Pauke abgedichtet werden Dazu Einführung eines Gummifingerlings, in dem eine stumpfe Kanüle eingebunden war in den Gehörgang. Der Gehörgang wurde mittels einer Spritze mit 2 ml Wasser von jeweils 0 C, 20 C oder 44 C gefüllt und der Temperaturverlauf in Abständen von 10 sec kontrolliert Nach den Messungen — im Durchschnitt 6 Messungen an einem Patienten — Weiterführung der jeweiligen Operation Bei den Antrotomien wurden die Messungen am 4 bis 6 Tag post operationem und nach Abklingen der entzündlichen Erscheinungen vorgenommen. Dabei erfolgte die Einführung der Meßsonde retroaurikulär Für die Temperaturmessung konnte nur ein Thermoelement verwendet werden, da die anatomischen Verhältnisse im Mittelohr naturgemäß eine flächenhafte Temperaturmeßsonde nicht gestatten Zum anderen mußte die Meßgenauigkeit groß sein, weil Temperaturdifferenzen bis 0 01 C registriert werden sollten Als Thermosonde fungierte ein Differentialthermoelement, aufgebaut aus Kupfer und Konstantan, mit einem Drahtdurchmesser von jeweils 0,1 mm. Die eine Lötstelle lag am Meßort während die andere konstant auf 20 C gehalten wurde Mit einem Multiflex-Galvanometer (Empfindlichkeit 4×10^{-9} A mm Widerstand $Ri = 1{,}3$ kΩ) konnte die Thermospannung gemessen werden Die Ablesegenauigkeit betrug 0 05 C.

ERGEBNISSE

Bei 11 Patienten wurden die operativen Eingriffe vorgenommen Sanierende Radikaloperation 4 11 Tympanoplastik 4 11 und Antrotomie 3 11 Dabei führten wir insgesamt 57 Messungen durch 1 mal Spülungen mit 0 C Wassertemperatur 25 mal mit 20 C und 15 mal mit 44 C. Die Ergebnisse der 3 Gruppen sind

Tabelle 1 *Temperaturmessungen am horizontalen Bogengang bei thermischer Reizung mit 20 C Wassertemperatur und Einwirkungszeit von 20 sec.*

1 Gruppe: Versuche 1 16, Radikaloperation, Tympanoplastik. 2. Gruppe Versuche 17–25, Antrotomie.

Versuch N	Zeit nach Wasserfüllung des Gehörganges 0"	10"	20"	30"	40"	50"	60"	2'	3'	4	5
1	0	0,3	0,8	1,2	1,0	0,8	0,6	0			
2	0	1,0	1,6	1,5	1,4	1,0	0,8	0			
3	0	0,1	0,2	0,2	0,2	0,2	0,2	0,2			
4	0	0,1	0,2	0,2	0,3	0,3	0,3	0,2			
5	0	0,2	0 4	0,4	0,2	0,1	0,1	0			
6	0	0,4	0,4	0,4	0,4	0,4	0,3	0			
7	0	0,2	0,4	0,6	0,5	0,4	0,3	0			
8	0	0	0,7	1,3	1,1	1,0	0,9	0,3			
9	0	0,4	0 6	0,7	0,7	0,6	0,6	0,4	0,3		
10	0	0,3	1,8	2,2	2,1	1,9	1 7	0,4	0,2		
11	0	0,5	1 4	2,0	1,8	1,0	0,6	0 3			
12	0	0,3	0,5	1,1	1,1	1,0	0,9	0,4	0,1		
13	0	0	0	1 6	1,3	1,1	0,7	0,3	0,1		
14	0	0,1	0,2	0,3	0,4	0,5	0,4	0,3	0,1		
15	0	0,3	0,5	0 7	0,6	0,6	0,4	0 4	0,4		
16	0	0 4	0,8	1,4	1,6	1,8	1,5	1,1	1,0		
Mittlere T-Änderung in °C		0,3	0,65	1,06	0,92	0,78	0,64	0,3	0,3		
17	0	0	0,1	0,2	0,3	0 4	0,4	0,3	0,1	0	
18	0	0	0,1	0,1	0,2	0 3	0,3	0,2	0,1	0	
19	0	0	0,1	0,2	0,2	0,3	0,3	0,2	0	0	
20	0	0,1	0,1	0,2	0,2	0,2	0,3	0,3	0,3	0,3	0,3
21	0	0	0 1	0,1	0,1	0,1	0 1	0,1	0,1	0,1	0,1
22	0	0	0,1	0,1	0,2	0,2	0,3	0,3	0,3	0,3	0,2
23	0	0	0,1	0,1	0,2	0,2	0 4	0,4	0,4	0,3	0,3
24	0	0	0,1	0,1	0,1	0,2	0,3	0,3	0,3	0,3	0,2
25	0	0	0	0,1	0,1	0 2	0,2	0,2	0,2	0,1	0,1
Mittlere T Änderung in C		0	0,09	0,13	0 18	0,25	0,29	0,25	0,2	0,16	0,13

1 Temperaturveränderungen am Bogengang bei thermischer Reizung mit 20 C Wassertemperatur

Es liegen 25 Messungen vor die im Durchschnitt eine Abkühlung am Bogengang von 0,8 C aufwiesen. Dabei differiert das Temperaturgefälle von maximal 2,8 C bis minimal 0,1 C (Tabelle 1)

Die Abkühlung hat bereits nach 20 bis 60 sec, im Durchschnitt nach 40 sec, den tiefsten Punkt erreicht. Die jeweils niedrigsten Werte sind in der Tabelle unterstrichen. Der Wiederanstieg bewegt sich in einem längeren

Tabelle 2 *Temperaturmessungen am horizontalen Bogengang bei thermischer Reizung mit 44 C Wassertemperatur und Einwirkung von 90 sec*

1 Gruppe: Versuche 1-9, Radikaloperation, Tympanoplastik 2. Gruppe: Versuche 10-16, Antrotomie

Versuch Nr.	Zeit nach Wasserfüllung des Gehörganges									
	0"	10"	20"	30"	40"	50"	60"	2'	3'	4'
1	0	0,1	0,1	0,1	0 1	0,2	0,2	0,2		
2	0	0	0	0	0,1	0,1	0 1	0,1		
3	0	0	0,1	0,1	0,1	0,2	0,3	0,2		
4	0	0,2	0,4	0,4	0,4	0,1	0,4	0,1		
5	0	0 1	0,6	0,5	0,6	0,6	0,6	0 6		
6	0	0,2	0,3	0,2	0,2	0,2	0,2	0,2		
7	0	0,3	0 4	0,5	0,6	0,6	0,6	0,6		
8	0	0 1	0,2	0,2	0,3	0,3	0,3	0,3		
9	0	0	0	0	0	0,1	0 1	0 1		
Mittlere T Änderung in C		0 14	0,25	0,22	0,23	0,3	0,3	0,3		
10	0	0	0	0	0	0	0,1	0,1	0	
11	0	0	0	0	0,1	0,1	0,1	0,1	0 1	
12	0	0	0,1	0,2	0,2	0,2	0,3	0,3	0,2	0 1
13	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0 1	0,1	0,2	0,1	0	
16	0	0	0,1	0,2	0,2	0,2	0,1	0 1	0	
Mittlere T Änderung in C		0	0,05	0,00	0,00	0,09	0 11	0 1	01	001

Zeitraum (2 bis 5 min) im Durchschnitt 3 min Nicht immer auch nach Registrierung bis zu 10 min, stellte sich die Ausgangstemperatur wieder ein In der Tabelle 1 weichen die Ergebnisse der Versuche 1 bis 16 und 17 bis 25 voneinander ab. Die obere Gruppe enthält die Radikaloperationen und Tympanoplastiken die untere die Antrotomien Letztere M sungen geben ein realeres Bild da durch Wegfall der Antrumbohrung und Erhalten der häutigen und knöchernen Gehörgangswand die Bedingungen weit physiologischer sind Hier registrierten wir eine durchschnittliche Abkühlung von 0 3 C nach 55 sec Bei den Versuchen konnte ein Nystagmus unter der Frenzel Brille nicht immer beobachtet werden Durch die horizontale Lagerung der Patienten trat im Vergleich zur Optimum t llung im Liegen mit Kopfstellung von 30 eine Hemmung auf

2. Temperaturmessungen am horizontalen Bogengang bei thermischer Reizung mit 44 C Wassertemperatur

Bei insgesamt 15 Messungen trat im Durchschnitt eine Erwärmung von 0,21 C auf (Tabelle 2)

Die Temperaturveränd rungen beginnen nach 10 sec und err ich n ihren

Tabelle 3 *Temperaturmessungen am horizontalen Bogengang bei thermischer Reizung mit 0° Wassertemperatur und Einwirkungszeit von 20 sec*

1. Gruppe Versuche 1–7 Radikaloperation, Tympanoplastik 2. Gruppe Versuche 8–17 Antrotomie.

Versuch Nr.	Zeit nach Wasserfüllung des Gehörganges 0″	20″	40″	60″	80″	100″	2′	3′	4′	5′
1	0	0,3	[illegible]	0,4	0,4	0,4	0,4	0,2		
2	0	0,3	0,4	0,5	[illegible]	0,6	0,6	0,4		
3	0	1,6	[illegible]	2,2	1,8	1,4	1,2	0,7		
4	0	2,5	[illegible]	2,5	1,7	1,1	0,8	0		
5	0	[illegible]	3,5	2,4	1,7	1,2	0,7	0		
6	0	0,8	[illegible]	1,1	1,0	0,9	0,7	0,5		
7	0	0,5	[illegible]	1,3	1,2	1,0	0,8	0,6		
Mittlere T-Änderung in °C		1,41	1,08	1,40	1,2	0,95	0,74	0,34		
8	0	0	0,2	0,5	[illegible]	0,5	0,4	0,2		
9	0	0,1	0,1	0,3	0,3	[illegible]	0,4	0,4	0,3	0,2
10	0	0,1	0,2	0,4	0,5	[illegible]	0,6	0,6	0,5	0,4
11	0	0,1	0,2	0,4	0,6	[illegible]	0,7	0,6	0,4	0,3
12	0	0	0,2	0,3	[illegible]	0,4	0,4	0,3	0,2	0,1
13	0	0,1	0,3	0,6	0,7	[illegible]	0,8	0,8	0,5	0,5
14	0	0,1	0,2	0,3	0,4	[illegible]	0,5	0,5	0,4	0,3
15	0	0,3	1,0	1,3	[illegible]	1,4	1,4	1,2	1,0	0,6
16	0	0,5	0,7	0,8	[illegible]	0,9	0,8	0,7	0,6	0,5
17	0	0,1	0,7	1,0	[illegible]	1,1	1,0	1,0	0,9	0,8
Mittlere T-Änderung in °C		0,14	0,28	0,50	0,7	0,73	0,7	0,61	0,48	0,37

Durchschnittswert nach 40 sec. Ein deutliches Wiederabsinken ließ sich in einigen Fällen nach 3 bis 4 min nachweisen. Ein Nystagmus konnte in allen Untersuchungen nicht beobachtet werden. Auch hier umfaßt die 2. Gruppe die Antrotomien (Versuch 10 bis 16) die ebenfalls mit dem Mittelwert von 0,13° C eine geringere Temperaturdifferenz haben.

3. Temperaturmessungen am horizontalen Bogengang bei thermischer Reizung mit 0° C Wassertemperatur

Zur Auswertung kamen 17 Messungen, die im Durchschnitt einen Temperaturabfall von 1,2° C aufwiesen. Die Werte liegen zwischen 3,6° C und 0,4° C (Tabelle 3).

Die Abkühlung hat nach 40 bis 100 sec, im Mittel nach 70 sec, den tiefsten Punkt erreicht. Eine Rückkehr zur Ausgangstemperatur trat selbst nach 5 min in der Regel nicht ein, jedoch stieg die Kurve ab der 2. min deutlich an.

In der Tabelle 3 sind ebenfalls die Messungen bei antrotomierten Patienten gesondert aufgeführt (Versuche 8–17). Die maximale Abkühlung

Tabelle 4 *Temperaturdifferenzen am Bogengang bei Nystagmusbeginn*

	Nystagmusbeginn		Nystagmusende	
Versuch	Zeit in sec	Temp.-Diff.	Zeit in sec	Temp. Diff.
1	30	0,1 C	180	0,2 C
2	30	0,1 C	60	0,5 C
4	60	2,1 C	180	0 C
5	10	2,6°C	120	0,7 C
6	30	1 1 C	180	0,5 C
10	50	0,3 C	180	0 6 C
13	60	0,0 C	180	0,6 C

schwankte zwischen 0 4 und 1 2 C, der Mittelwert liegt bei 0 7, C und einer Zeit von 90 sec.

Nystagmus trat nur in 7 Fällen auf der Grund hierfür liegt in der erwähnten nicht optimalen Kopfhaltung im Liegen. Das Einsetzen des Nystagmus scheint in keinem direkten Zusammenhang zur Abkühlung am Bogengang zu stehen (Tabelle 4) Im Durchschnitt begann der Nystagmus nach 40 sec und erlosch nach 2,5 min

DISKUSSION DER ERGEBNISSE

Sicherlich entsprechen unsere Versuchsbedingungen durch die Unzugänglichkeit der operativen Eingriffe nicht ganz den physiologischen Verhältnissen. Bei der 1 Gruppe (Radikaloperation und Tympanoplastik)

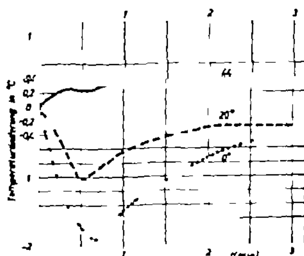


Abb 1 Gegenüberstellung der Kurve ... bei thermischer Reizung mit ... 44°C und 0 C Wassertemperatur (Durchschnittswert der 1 Gruppe Radikaloperationen, Tympanoplastik n.)

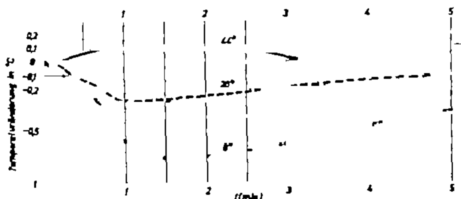


Abb. 2. Gegenüberstellung der Kurvenverläufe bei thermischer Reizung mit 20°C, 44°C und 0 C Wassertemperatur (2. Gruppe A trotomien.)

kommt es zu Veränderungen im Gehörgang, wenn auch der perilabyrinthäre Block erhalten bleibt Bei der 2 Gruppe (Antrotomie) ist das Zellsystem am Antrum verändert. Letztere Versuchsreihe scheint auf Grund des erhaltenen Gehörganges den natürlichen Verhältnissen eher gerecht zu werden, wofür auch die einheitlicheren Meßergebnisse dieser Gruppe sprechen

Allen Messungen ist eine stärkere zeitliche Änderung der Temperatur in der Anfangsphase gegenüber der anschließenden Normalisierungsphase gemeinsam. Die Durchschnittsergebnisse der beiden Gruppen in graphischer Darstellung

Im Kurvenverlauf kommt zum Ausdruck, daß die Zeit bis zum Maximum bzw Minimum kürzer ist als die vom Extremum bis zur Rückkehr der Ausgangstemperatur

Der Abfall der Temperatur um 0 1 C bei Eiswasserreizung (Durchschnittswerte bei der Antrotomiegruppe)vollzieht sich in 10 sec, während die Wiedererwärmung um 0 1 C in 60 sec geschieht Die Zeiten verhalten sich wie 1 6

In Rücksichtnahme auf die Patienten konnte die völlige Normalisierung der Temperatur nicht immer abgewartet werden Es lassen sich jedoch Richtwerte für die Erholzeiten angeben

5 min bei 44 C — Spülung
8 min bei 20 C — Spülung
10 min bei 0 C — Spülung

Diese Zeiten müßten für aufeinanderfolgende thermische Reizungen Berücksichtigung find n, auch wenn der auftretende Nystagmus weitaus eher abklingt.

Die Messungen zeigen daß die Temperaturveränderungen sich zum thermischen Reiz adäquat verhalten

Zwischen der Korpertemperatur (37 C) und der Temperatur der Spulflüssigkeit bestehen folgende Temperaturunterschiede am Bogengang

7 C (Warmwasserspülung) — 0,21 C
17 C (Kaltwasserspülung) — 0,8 C
37 C (Eiswasserspülung) — 1,2 C

Für die Frage bei welcher Temperturveränderung am Bogengang Nystagmus einsetzt reicht die Anzahl der Versuche mit Nystagmus nicht aus (Tabelle 4) Der Nystagmusbeginn lag bei minimal 0,3 C und maximal ? 0 C. Das Aufhoren des Nystagmus zeigte sich einheitlicher bei ungefähr 0,6 C. Die Rolle des Gummifingerlings als isolierende Schicht im Gehorgang ist — wie Vergleichsmessungen mit und ohne Fingerling bei den antrotomierten Patienten ergaben — *unbedeutend*

Im Vergleich mit den Ergebnissen von Schmaltz & Vögler (2 Versuche) *weichen unsere Messungen erheblich ab. Die Abkühlung* am horizontalen Bogengang ist bei unseren Eiswasserspülungen (Antrotomien) um das Doppelte größer Genaue zeitliche Angaben bei den genannten Autoren fehlen leider Der Kurvenverlauf mit steilerem Anstieg und langsamen Abfall ist ähnlich dem unsrigen.

SUMMARY

Only two cases exist of temperature recordings of the horizontal semicircular canal on *living human-beings after thermal stimulation. During 11 operations* an electrical recording instrument was placed in the semicircular canal through the auditory canal, by radical operation and tympanic plastic operation or retroauricular by *antrotomy and altogether 57 temperature recordings after thermal* stimulation (filling of the auditory canal with 5 ml water for 70 sec) registered. Twenty five recordings of a 20 C stimulation showed an average supercooling *of 0.8 C after 40 sec 15 recordings of a 44 C stimulation showed a temperature* rise of 0. 21C after 40 sec, and 17 recordings during stimulation at 0 C after 0 sec In all cases, at the beginning, there was a quick change of temperature *up to a maximum and then a slow return to the initial temperature (relation* of 1 0) The results from the above showed recovery times of ... un ... stimulation at 44 8 min at 20 and 10 min at 0 C Nystagmus was seen only *in cases during flushing with cold water because the examination was d e in a* lying position No direct connection with the change of temperature on the ... circular canal was shown. The separate values of the antrotomy group shown *on the table are more exact because of the more physiological conditions of* examinations.

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CUPULA CUPULAR ZONE OF OTOLITHIC MEMBRANE AND TECTORIAL MEMBRANE IN THE SQUIRREL MONKEY

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The microscopic architecture of the cupula was studied in subhuman primates. It was determined that the cupula has a honeycomb-like meshwork and that this meshwork may contain both neutral and acid polysaccharides, as well as mucoprotein. The nature of the contents in the space enclosed by this meshwork is not certain although the existence of a small amount of acid mucopolysaccharide is suggested. The cupular zone of the otolithic membrane was stained both by PAS and alcian blue similar to the cupular meshwork, but the subcupular zone was not stained either in the cupula or the otolithic membrane. The border of *the tectorial membrane reacted very strongly to both PAS and alcian blue* stainings. Within the tectorial membrane the contents reacted moderately. The fibrils in the tectorial membrane seemed to be stained darker than the surrounding components, suggesting neutral and acid mucopolysaccharides and mucoprotein. These findings suggest that the meshwork of the cupula, the cupular zone of the otolithic membrane and the tectorial membrane have basically identical chemical components.

The so-called gelatinous or jelly-like substances in the inner ear end organ apparatus are always located close to the top of the hairs of the sensory cells and are believed to have a very important role in creating the shearing force between these substances and the hair cells. The purpose of the present study is to investigate the microscopic architecture of the cupula of the crista ampullaris in squirrel monkeys and to attempt to determine the chemical components of the gelatinous substances of the cupula of the crista ampullaris, cupular zone of the otolithic membrane and tectorial membrane by using special staining techniques, thereby contributing further information to previous descriptions.

METHOD

Twenty micron thick celloidin sections of squirrel monkey temporal bones which were originally prepared for light microscopic investigation were

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decelloidinized, mounted on glass slides and stained in periodic acid Schiff solution and in alcian blue (Steedman, 1950)

In addition to these sections with special stains, many hematoxylin eosin stained sections were studied to investigate the cupulae in several planes and from different angles

FINDINGS

Cupula

As seen in Fig 1 one hematoxylin eosin stained cupula, cut at near cross-section plane, demonstrates the existence of a canalicular architecture for the squirrel monkey cupula. This histological configuration was previously suggested by Wittmaack (1935) Vilstrup (1950) Wersäll (1956) Dohlman *et al.* (1959) in other animals. When the cupula is cut sagittally the mesh work of this canalicular structure appears as fine filaments or fibers. In the partly collapsed view the spaces of the microcanals look wider than the meshwork substance, especially at the lower part of the cupula. Technical artifacts cannot be completely ruled out in these preparations however a similar relationship was also noticed in the well extended cupula.

The insertions of the sensory hairs into these microcanals could not be demonstrated in the alcian blue stained section (Fig. 3) thus it will still leave the question open whether the tips of the sensory hairs are inserted in the microcanals or attached to the walls of the meshwork however in some areas, the location of the tips of the hairs suggests that they might extend into the cupular microcanals. Similar findings were noticed in PAS stained cupulae (Fig 2)

The side walls of these microcanals, which constitute a meshwork (previously described as filaments) were strongly PAS positive as has been described by som researchers (Wislocki & Ladman, 1954 1955 Reinecke *et al.*, 1960 Belanger 1956 *a* Iurato, 1967) as well as alcian blue and hematoxylin however the contents of the microcanals are very lightly stained, or not stained at all in these sections.

The basement membrane, which is related to the mucopolysaccharide ground substance below the supporting cells is stained strongly both by PAS and alcian blue

Otolithic Membrane

The cupular zone of the otolithic end organs is strongly stained in PAS and moderat ly so in alcian blue in these specimens.

In the present light microscopic investigation the subzone of the cupular zone of the otolithic end organs, i n t identified. The basement membrane below the macular supporting cells was also stained both in PAS and in alcian blue. In Fig 4 the cupular zone extends to the edge of the macula while in Fig 5 the subcupular zone seems to open to the endolymphatic space



Fig 1 Photomicrograph d monstrates the honeycomb-like canalicul r rchitect re f the sq irrel mo key p l H mat lin-Eosi st i i g 310

The rod shaped structures of the cupular zone of ot lithic m mbrane which are frequently seen as fibers light microsc picall J hnss n & Haw kins, 1967 Igara hi, 1966 1967) are larger than sensory hairs th mselves and are definitely not direct extensions f the sens ry hairs.

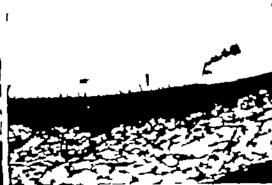
Tectorial Membrane

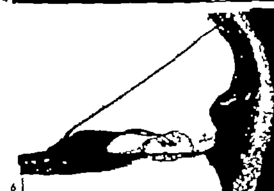
The tectorial membranes were also stained b th in LAS (C n sen, 1966 and in alcian blue (Figs. 6 and) The b rder f the tect rial m mbrane l











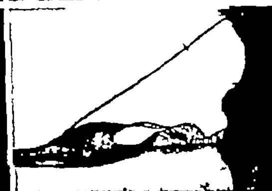


Fig 2. Photomicrograph exhibit the PAS-positi cupula filament (meshwork) i squirrel monkey cupula. PAS stai ing. 270.

Fig 3. Photomicrograph demo strates the alcia bl stained cupular filaments (meshwork) i squirrel monkey cupula. The basement membrane is lso stained. Alci blue staining 330.

Fig 4 Photomicrograph demonstrates the PAS-positi cupula zone of otolithic membrane (squirrel mo key) Note the basement membrane i lso stained. PAS stai i g. 300

Fig 5 Photomicrograph exhibits the alcia blue stained cupul zone from squirrel monkey otolithic membrane Alcia blue staining. 300.

Fig 6. Photomicrograph demonstrates the PAS-stained organ of Corti nd surroundi g components. Tectorial membrane i positiv ly stained by PAS PAS stai i g. 130

Fig 7 Photomicrograph exhibit the lcia blue stained orga f Corti nd surrostruct res. Tectorial membrane i stained by lcia blue Alcia bl staining.

more darkly stained with both stains than are the contents. The filaments (Iurato, 1960) are not clearly recognizable in the present light microscopic investigation however they seem to be stained darker than the other components of the tectorial membrane as was described by Vinnikov & Titova (1964)

The cuticular plates of the crista, macula, and organ of Corti were moderately or strongly stained by PAS and alcian blue as was the basement membrane in the crista ampullaris, the macula, and the cochlear duct.

In all specimens, the limbus spiralis and the spiral ligament were also stained both by PAS and alcian blue The hair cells in the organ of Corti stained slightly to moderately both with PAS and alcian blue. The infra nuclear area of the outer hair cells was stained especially darker The stria vascularis and the secretory epithelium of the vestibule were stained both by PAS and alcian blue to some extent

DISCUSSION

The cross section view of the cupula, especially at the central portion close to the crista, demonstrates a honeycomb-like architecture Iurato (1967) has suggested that these meshes correspond to borders of supporting and sensory cells however this could not be identified in the present light microscopic study

Dohlman *et al.* (1959) noted that the cupula seemed to be made up of crossing fibers, with an interposed interfibrillar substance which showed no microscopic structure The fibers revealed a strongly positive PAS reaction and the interfibrillar substance was stained pink. They also noted that the cupulae are constructed as a meshwork, presumably of collagen fibers, with certain orientation, and that endolymphatic fluid, containing sulfo-mucopolysaccharide, filled the interfibrillar spaces of this meshwork.

The present studies confirm that this meshwork strongly reacted to both PAS and alcian blue, and also that the contents of the micro-canal spaces may contain some of the chemical components which are found to take up these stains. The results, however raise a question about the chemical composition inside the microcanals of the cupula. It could be mucopolysaccharide If these microcanals open directly to the subcupular zone, then the contents of the microcanals of the cupula and of the subcupular zone sh uld be somewhat identical. The ch mical components of the subcupular zone is not kn wn It is not known if the subcupular zone (Vilstrup, 1950) is freely open to the endolymphatic space or not. Furthermore, it is not known if the opposite end of the cupula opens into the endolymphatic space (Wersäll 1956) or not.

The fibrils of the cupula, which are actually the meshwork structure of the cupula are unlikely to be continuous with sensory hairs. In further support of this, it has been found previously that the rod-shaped structure

in the cupular zone of the otolithic membrane was not an extension of the sensory hairs.

Vilstrup (1950) made an extensive investigation of the cupula treated by the freeze-drying method in many different species of fish. He demonstrated the clear existence of fibrils in the cupula and believed that no structure was present between these fibrils. This finding was confirmed by the present investigation.

Wislocki & Ladman (1954) suggested absence of sulfated (acid) mucopolysaccharide in the filaments of the cupula; however, the present investigation as well as that of others (Dohlman *et al.*, 1959; Jensen & Vilstrup, 1960) suggests that the meshwork of the monkey cupula contains both neutral and acid mucopolysaccharide as well as mucoprotein.

The cupular zone of the otolithic end organs is strongly stained in PAS and moderately so in alcian blue. This finding suggests that this cupular zone contains both acid and neutral mucopolysaccharide as well as mucoprotein as also found in the meshwork of the cupula. The electron microscopic structure of the cupular zone of the otolithic membrane has been previously reported (Igarashi & Kanda, 1968). Although comparison of the rod shaped structure in the cupular zone of the otolithic membrane with the meshwork material of the cupula reveals that these structures do not seem to be identical morphologically, the possibility exists that these two structures have similar chemical composition.

Plotz & Perlman (1955) have made a detailed morphological and histochemical study of the bat cochlea after fixation by freezing and drying. They found that the fibers of the tectorial membrane stained deeply with the Hotchkiss method. A phase contrast microscopic investigation of these tectorial fibers has been performed by Engstrom (1951), Hilding (1952) and Katsuki & Covell (1953). These fibrils seem to be extremely thin (Smith, 1955; Engstrom & Wersäll, 1958) and it is known that these are not extensions of sensory hairs when electron microscopic investigations of sensory hair tectoria attachment have been done (Iurato, 1961; Engstrom *et al.* 1962; Spoendlin, 1966; Kimura, 1966). Although the fibrils are not clearly seen, findings here suggest that the tectorial membranes contain both acid and neutral mucopolysaccharide as well as mucoprotein.

The autoradiographic studies of radiosulfate incorporation in the inner ear (Belanger, 1953) revealed a relatively high uptake by the tectorial membrane and also by the gelatinous mass of the cupula. Therefore it seems evident that the inner ear end organ structures contain a large amount of polysaccharides and that these are at least partly sulfated, because the radiosulfate was retained as newly synthesized sulfo-mucopolysaccharide. According to another study by Belanger (1956), the tectorial membrane showed the presence of highly polymerized saccharides reacting to the PAS staining method.

Although the findings in the meshwork of cupula, cupular zone of the otolithic membrane and the tectorial membrane suggest that these true

tures contain a similar chemical component which is neutral and acid mucopolysaccharide and mucoprotein morphological differences are found. The overall chemical similarities in spite of morphological differences suggest that all of these structures may have similar embryological origin

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ZUSAMMENFASSUNG

Es wurde die mikroskopische Architektur der Kupula in subhumanen Primaten untersucht. Dabei wurde festgest llt, dass die Kupula ein H nigwaben — äh liches Netzwerk hat und dass dieses Netzwerk sowohl neutrale und saure Poly saccharide als auch Mucoproteine enth llen könnte. Die Beschaffenheit dieses von diesem Netzwerk umschlossenen Inhalts ist nicht gewiss, es wird aber die An wesenheit ines geringen Betrages an sauren Mucopolysacchariden angen mmen. Die kupulare Zone der tolithischen Membran zeigte Farbung mit PAS und Elsass-Blau, ähnlich dem kupularen Netzgewebe, jedoch war die subkupulare Zone sowohl in der Kupula als uch in der otolithischen Membran nicht gefärbt. Di Grenze der tektorial Membran reagierte sehr stark mit PAS und Elsass-Blaufarbung. Innerhalb der tektoriale Membran war die Reaktion des Inhalts mässig. Die Fibrillen in der t kt rial Membran scheinen dunkler gefärbt zu werden als die umgebenden Bestandteile, was uf neutral und saure Mucopolysaccharide und Mucoprot in schliessen lässt Diese Fund lassen annehmen, dass das Netz werk der Kupula, die kupulare Zone der otolithischen M mbran und d tektoriale Membran fundamental di gleichen hemischen Komponenten haben.

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A COMPARISON OF THE EFFECTS OF (–) HYOSCINE AND AMYLOBARBITONE SODIUM ON MEASUREMENTS OF POST ROTATIONAL TURNING SENSATION AND NYSTAGMUS

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Following the reports of ea lier workers an experiment was carried out to assess the possible value f cupulometric techniques both in lucidat ing the mechanism of tion f anti-motion sickness drugs and in screening ew drugs for possible a ti-motion si kness effect. The drugs used were (–)-hyoscine d amylobarbitone sodium because although both have central depressa t properties in commo nly (–) hyoscine has been sh wn t be consistently effective against motion si kness.

There was some evidence t suggest that (–) hyoscin shortened the durati n of post-rotational turning sensation and that both drugs reduced th time constant of decay of post-rotational nystagmus. However both these effects could well be attributable t generalised depressi n of th central nervous system. Any more specifi pharmacological tion was bscured both by habituation ffects and idiosyncrasy of indi idual response these factors render such tests f doubtful value in the screening of nti motion si kness drugs.

Alth ugh there is now substantial evidence for the effectiveness of single oral doses of (–) hyoscine in the prophylaxis of motion sickness induced by xposure to motion of short duration (Brand & Perry 1966) the mechanism of action of the drug remains obscure It has, however been shown that certain laboratory tests of labyrinthine function (post rotational turning sensation and nystagmus) may be modified by the prior administration of (–) hyoscine (Boehen k & Ormerod, 1962 Benson & Brand, 1968) It has also been reported that other drugs which are known to protect against motion sickness (e.g. diphenhydramine promethazine and cinnarizine) have similar properties (Gutner *et al* 1951 Philipszoon, 1962 de Wit, 1953 Bortalena, 1955 Aschan, 1967) Since the presence of an intact labyrinthine system is essential for the production of motion sickness (Kennedy *et al* 1965) it was con idered that the modification of laboratory tests of labyrin thine function might provide the basis for a screening test for anti-motion sickness drugs and also afford a clue as to their mechanism of action However it remained uncertain whether modification of the effects of stimula tion of the labyrinth could be attributed to any specific anti-motion sickness

property of the drugs or whether they were merely manifestations of a central depressant effect further there was evidence to suggest that post rotational nystagmus might also be modified by drugs which have central depressant properties but no anti motion sickness activity (Buchenek & Ormerod, 1962) In an attempt to clarify this point an experiment was carried out to compare the post rotational responses of subjects who had received either (−) hyoscine 0 40 mg or amylobarbitone sodium 200 mg with a placebo

METHODS

The methods used were similar to those described previously (Benson & Brand, 1968 Brand 1968) Using a turning chair each subject received impulsive decelerative stimuli from constant angular velocities of 60 20 1 and 7 ./sec in both anti-clockwise and clockwise directions. The duration of the subjective post rotational turning sensation was timed after each impulse and post-rotational nystagmus was recorded using a conventional electronystagmographic technique

The subjects who took part in this experiment were 15 sailors with no previous experience of rotational test each was tested at the same time of day on three consecutive days. One hour before the test the subject swallowed a capsule which contained either (−) hyoscine base 0 40 mg, amylobarbitone sodium 200 mg or a lactose placebo the drugs were allocated according to a balanced randomised design and the experiment was carried out under double-blind conditions The experimental findings were examined by analysis of variance

RESULTS

Post rotational Turning Sensation

The mean duration of subjective post-rotational turning sensation obtained from all subjects after each impulsive deceleration on each day of the experiment have been plotted in the form of the sensation cupulogram of van Egmond *et al* 1948) a Fig 1 The decline in response due to habituation which occurs with iteration of tests of this nature is clearly shown and is comparable in magnitude to that which was observed in earlier experiments (Brand, 1968

These data have been sorted into treatment groups and are plotted as sensation cupulogram in Fig. 2 Although the mean responses obtained from the subject group after hyoscine appear to be somewhat shorter than after the placebo or amylobarbitone sodium when analysis was carried out it was not possible to demonstrate any significant difference between the two drugs and the placebo at the 5 level of confidence

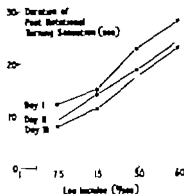


Fig. 1

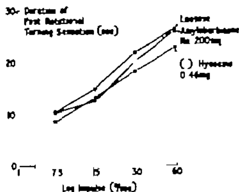


Fig. 2

Fig. 1 The effect of habituati n on the sensation cupulogram. Duration of post-rotation l turning sensation (sec) fter impulsive deceleratio from th constant angular locities used on each day f the experiment (N = 15)

Fig. 2 The effect f (−)-hyoscine nd amylobarbitone sodi m n the sensation cupulogram.

Post rotational Nystagmus

Records of the post-rotational nystagmus engendered by impulsive deceleration from a constant angular velocity of 60 /sec were obtained from each subject over a period of 30 sec. These were examined by measuring the angular velocity of each beat of the slow phase and plotting it on a log. scale as a function of time after deceleration An indication of the rate of decay of the labyrinthine signal is then afforded by an examination of the slope of the regression line drawn through these points, (time constant of decay van Egmond & Groen, 1955 τ/Δ Benson, 1967) and the intercept of the extrapolated regression line on the ordinate provides an estimate of the initial angular velocity of the slow phase Although plots of the angular velocity of the slow phase of nystagmus as a function of time are usually linear when a logarithmic scale is used for the ordinate, in three of the records the nystagmus did not decay as a simple exponential function and therefore could not be included in the analysis Time constants of slow phase decay were calculated from the records of the remaining 12 subjects and these are set out in Tabl 1 The normal range of such values has been given as 6.25 to 24 sec with a mean of 13 sec (Benson, 1967) and the present observations fall reasonably well within this grouping It can be seen that the mean alues which were obtained after both (−) hyoscin and amylobarbitone are closely similar but that both are smaller than those obtained after the placebo This finding is significant at the 5% level of confidence and indicates that the effect of both drug was to increase th rate of decay of the nystagmic response engendered by impulsive deceleration from a con tant angular elocity

properties of the drugs or whether they were merely manifestations of a central depressant effect further there was evidence to suggest that post rotational nystagmus might also be modified by drugs which have central depressant properties but no anti motion sickness activity (Bochenek & Ormerod 1969) In an attempt to clarify this point an experiment was carried out to compare the post rotational responses of subjects who had received either (−) hyoscine 0 46 mg or amylobarbitone sodium 200 m with a placebo.

METHODS

The methods used were similar to those described previously (Benson & Brand 1968 Brand, 1968) Using a turning chair each subject received impulsive decelerative stimuli from constant angular velocities of 60, 30 15 and ·5 /sec in both anti-clockwise and clockwise directions The duration of the subjective post rotational turning sensation was timed after each impulse and post rotational nystagmus was recorded using a conventional electronystagmographic technique

The subjects who took part in this experiment were 15 sailors with no previous experience of rotational tests each was tested at the same time of day on three consecutive days. One hour before the test the subject swallowed a capsule which contained either (−) hyoscine 1 a e 0 46 mg amylobarbitone sodium 200 mg, or a lactose placebo the drugs were allocated according to a balanced randomised design and the experiment was carried out under double-blind conditions The experimental findings were examined by analysis of variance

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These data have been sorted into treatment groups and are plotted as sensation cupulograms in Fig 2 Although the mean responses obtained from the subject group after hyoscine appear to be somewhat shorter than after the placebo or amylobarbitone sodium when analysis was carried out it was not possible to demonstrate any significant difference between the two drugs and the placebo at the ·5 level of confidence

metic did not bring about any significant change in the rate of decay of nystagmus from that obtained at the beginning of the test session

The point of interception of the extrapolated regression lines on the ordinate (ωt_0) was also determined for each subject in each treatment group and the values which were obtained are set out in Table 2 there was however no significant difference between the mean values obtained on each day of the experiment nor between the treatment groups

DISCUSSION

Post rotational Turning Sensation

Although the means of the results obtained on each day of the experiment and from each treatment group have been plotted in the form of the sensation cupulogram" they have not been expressed as "slope values" since recent experimental evidence (Brand, 1968) suggests that the relationship between stimulus and response which is obtained in this manner is not linear for all subjects

Examination of Fig 1 shows that a considerable response decline occurred on each day of the test and this may be compared to the day effect" which was observed in a similar experiment when the test was repeated on nine consecutive days (Brand, 1968) This appears to be a further example of habituation or adaptation to a repeated stimulus.

When the experimental data are sorted into treatment groups and the mean values plotted, there is a suggestion that the effect of (−) hyoscine was to depress the response relative to that which was obtained when the same group of subjects was given a placebo This finding is in general agreement with those of an earlier experiment performed under similar conditions (Benson & Brand, 1968) when doses of 0.21 and 0 46 mg of (−) hyoscine given subcutaneously produced a well-defined shortening of post-rotational turning sensation. However analysis of variance showed that the difference observed in the present experiment is not statistically significant Although the same dose levels were used in both experiments it is probable that a more powerful effect would be obtained after parenteral administration. Further the subjects appear to differ considerably in their responses to hyoscine and it is this interaction which must be used to determine the overall drug effect. It is well known that idiosyncrasy of response is frequently observed afte the administration of (−) hyoscine for general clinical purposes and the effects noted in the present experiment may well be a further example of this property of the drug.

Post rotational Nystagmus

In ll subjects characteristic nystagmu with clearly-defined slow nd fast phases could be elicited after deceleration from constant angular velocities

of 60 /sec Detailed examination of the ω and τ/Λ values which describe the nystagmic response revealed no significant modification of nystagmus with repetition of the test. This may be contrasted with earlier observations which indicated that the effect of habituation on tests of this nature is to cause a fall in time constant of decay although measurements of extrapolated initial angular velocity remain relatively constant (Brand, 1968).

Neither drug was found to modify the initial angular velocity (ω) extrapolated from plots of slow phase decay against time. These findings are in general agreement with a study of the effect of barbiturates on post-rotational nystagmus made by Bochenek & Ormerod (1969), although these workers measured total duration, total number of beats and total amplitude of nystagmus and not time constants of slow phase decay. They reported that a single dose of 200 mg of amylobarbitone sodium given one hour before the test induced a depression of response comparable to that produced by 0.3 mg of (–) hyoscine.

In the present experiment the drugs caused no alteration of the normal pattern of nystagmus elicited by impulsive decelerations. This may be contrasted with the findings of a similar experiment when (–) hyoscine was administered subcutaneously in doses of 0.21 and 0.46 mg: this produced a profound degradation of the normal pattern of nystagmus, so that fast and slow phases could no longer be clearly recognised and were replaced by pendular eye movements (Henson & Brand, 1968). The normal nystagmic pattern could however be restored when the subject performed mental arithmetic and this was attributed to the increased level of arousal which has been shown to be associated with an increase in nystagmic output (Brand, 1965). In the present experiment the performance of mental arithmetic had no effect either on the gross appearance of the records or on the time constants of decay derived from them. This difference in effect of doses of the same size in the two experiments is probably attributable to the more complete absorption which is known to occur one hour after dose when the subcutaneous route of administration is employed (Tonndorf *et al.*, 1953), so that the present observations may well represent the action of a smaller effective dose of the drug. However, the mean values observed after both drugs are significantly smaller than those obtained from the same subject after administration of placebo. This indicates that the effect of the drugs was to increase the rate of decay of the nystagmic response, possibly by decreasing mental arousal: both drugs possess central depressant properties.

The findings of this experiment cast considerable doubt on the value of rotational tests of labyrinthine function for the demonstration of motion sickness properties of a given drug. They also underline the difficulties of assessing any drug which modifies clinical symptoms of labyrinth [illegible] by use of cupulometric techniques. Habituation phenomena are [illegible] sufficiently so powerful as to confound any more specific pharmacological effects, and the central depressant properties which such drugs commonly possess may modify nystagmus in their own right.

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ZUSAMMENFASSUNG

Früheren Berichten folgend, wurden Versuche durchgeführt, um den Wert des kupulometrischen Verf hrens zur Klarstellung des Aktionsmechanismus von Medikame ten gegen Seekrankheit und zur Prüfung neuer Medikamente uf ihre mögliche Wirkung gegen Seekrankheit bzuschätzen. Die Medikamente, di untersucht wurden, waren (−)-Hyoscl und Natrium-Amyl-Dyäthylbarbiturat, da, obwohl beid beruhigend auf das Zentral ervensystem wirken nur Hyoscin unter allen Umständen gegen Seekrankheit wirksam ist.

Es ergab si h Beweismaterial, das darauf hinwies, dass (−) Hyoscin die Dauer des nach R tati n stattfindenden Drehgefühls verkürzt und dass beide Medikamente die Zeitkonstante des Ablaufen des na h Rotation stattfindenden Augen zitterns reduzieren. Diese beiden Wirkungen könnten jedoch ebensogut auf verallgemeinert Depression des Zentralnervensystems zurückzuführen sein. Jed wesentlich pharmakologische Wi ku g wurde durch Gewöh ungserschei ungen und durch die Idiosynkrasie der individuellen Reaktion verschleiert. Diese Faktoren st llen den Wert sol her Versuche zur Prüfung von Medikamenten gegen Seekrankheit in Zweif l.

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POSTURAL VARIATIONS OF NASAL PATENCY

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Comparati e quantitativ measurements of the nasal airway resistance (Rn) in different positions were performed in normal individuals, in p tients with allergic rhinitis, and in patients with common colds during and after infection. In normal persons and in patients recovered from common colds Rn increased only very little in positions of 20° or less above the horizontal plane. In rhinitic patients the same positions were followed by a considerable increase of Rn caused by swelling of the nasal mucosa due to hydrostatic blood pressure i crease. This was proved by the effect of neck vein compression causing about the same in crease of Rn as the horizo tal position. The mucosal reactions to high transmural pressures support the theory of vascular hypotoni ity in the rhiniti nose. In iew of the biological and clinical consequences of the postural effects n th nasal mucosa, common colds should not be cured with bed rest t night, rhinitic patients should take up a body position f more than 20° abov the horizontal level.

Nasal stuffiness in the recumbent position is a well known experience during a common cold and is also a major complaint in patients suffering from all rgic or vasomotor rhinitis. The present author previously studied the variations of nasal airway resistance during different postures in pa tients with allergic rhinitis and found that a good many of these developed considerably increased resistance when lying down horizontally (Rundcrantz, 1964)

Many authors have previously reported on the postural effect on the nasal patency Lying on one side causes obstruction of the lower passage of the nose (Kayser 1895 Heetderks, 192) According to Vacher (1905) a "nasal insufficiency" is often seen in children in the recumbent position because the cavern us tissue of the nasal mucosa is engorged owing to gravity Interruption of sympathetic nerve impulses to the nose causes congestion of the nasal mucosa in the horizontal position (Maestranzi, 1927 Winslow *et al* 1934 Curcović 1937 Millonig *et al* 1950 Connor *et al* 1957 Hamberger 1961)

The aim of the present investigation is to study the reactions of the nasal mucosa to different postures in healthy individuals and in patients suffer ing from allergic rhinitis and common colds. The possible mechanisms and the biological importance of nasal patency will also be discussed.

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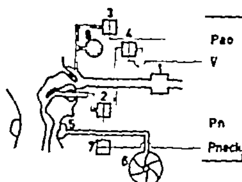


Fig 1 Block diagram f measuring device f r the det rmination f Rn. 1 – flow reg l t 2 – pre ure transducer for Pn; 3 – pressure transducer for Pa 4 – pressure transducer for V; 5 – eck in cuff 6 – electric f 1 flating the cuff; 7 – pres re tra sducer for Pneck 8 – mecha ic l m n meter howing Pao.

METHOD

Fig 1 is a block diagram of the measuring device used in the investigation The nasal airway resistance of the entire nose (Rn) was measured by means of the rhinomanometric method described by Ingelstedt *et al* (1960) This method is based upon an air flow regulator (Fig 1 1) which allows a pre-set constant airflow to pa s through the nasal cavities via a tight fitting nasal mask during spontaneous breathing The flow regulat r is described in detail by Jonson (1960) The constant flow is independent of the respiratory efforts of the patient as long as the pressure in ide the ma k exceeds 2 cm H_2O The differential pressure between the na al mask and the oral cavity—the pressure drop across the nose (Pn) was measured by a differential gas pressure transducer (EMT 33, Elema-Sch nander pressure range 0–30 mm Hg) (Fig 1 2)

The pressure inside the mask (Pao) reflecting the respirat ry efforts was measured by another transducer (EMT 33) (Fig 1 3) Thi pres ure could also be controlled by the subject on a mechanical manomet r (Fig 1 8) The pre-set airflow (V) was controlled via a pneum tachograph (Fleisch No 2) and a transducer (EMT 32, pressure range 0 1o m n H_2O) (Fig 1 1) An inflatable neck vein cuff of 6 cm width and 50 cm l n th wa also used (Fig. 1 5) It was inflated by an electric fan (Fig 1 6) th pres sure of which (Pneck) was mea ured by a transducer (EMT 3o pr sure range 0 300 mm Hg) (Fig 1 7) The ignal fr m the transduc wer amplified and recorded on a multi-ch nnel Mingograph 81 (El ma Sch nan der)

MATERIAL AND PROCEDURE

The entire material consisted f 28 subjects, 23 men and 5 w m n ed 14–41 They were divided into 4 gr ups

Table 1

Subjects	No.		$Rn_{90°}$	Rn_{30}	Rn_{20}	Rn_{0}	Rn_{C}	ΔRn
Group 1								
Healthy	10	Mean	1.4	1.6	2.0	2.1	2.2	Range
		S.D.	0.4	0.6	0.6	0.8	0.9	0–1.6
		S.E.	0.09	0.13	0.13	0.18	0.19	
Group 2								
Allergic rhinitis	10	Mean	3.1	3.4	5.6	8.5	6.5	0.8–5.0
		S.D.	1.6	1.6	3.3	4.6	4.0	
		S.E.	0.36	0.38	0.78	1.02	0.93	
Group 3								
Infectious rhinitis	8	Mean	2.7	3.5	4.9	7.7	5.0	1.0–3.2
		S.D.	1.3	1.7	2.9	4.4	3.3	
		S.E.	0.32	0.44	0.77	1.09	0.87	
Group 4								
After infection	7	Mean	1.4	1.9	2.6	3.0	2.6	0–0.6
		S.D.	0.4	0.4	0.7	0.9	0.9	
		S.E.	0.10	0.11	0.21	0.24	0.26	

Mean, standard deviation and standard error of mean of *Rn* in different positions and during neck vein compression (cm H_2O/LPS) V = 0.5 LPS. ΔR = change of R during prolonged inspiration.

(1) Healthy volunteers without any history or clinical signs of nasal disorders, 10 persons.
(2) Patients with allergic rhinitis, verified by intracutaneous tests and intranasal provocation but not yet submitted to treatment, 10 persons.
(3) Patients with acute infectious rhinitis (common colds) 8 persons.
(4) Seven of the patients from group 3 one month after recovery from infection

During the examinations the patients were instructed to blow their noses carefully and repeatedly. Each individual was examined on two separate occasions, groups 1, 2 and 4 with a few days interval, group 3 during one day with a few hours interval

The nasal airway resistance (Rn) was measured at an air flow (V) of 0.5 litres/sec (LPS). The subjects were instructed to make respiratory efforts producing a pressure in the nose mask of 5 cm H_2O positive and negative which they could check themselves on the mechanical manometer. Sitting in a folding chair the subject could be placed in different body positions. The mean values of Rn were calculated from five consecutive inspirations and expirations, performed during the following conditions

(a) sitting erect ($Rn_{90°}$)
(b) lying dorsally 30° [illegible] plane ($Rn_{30°}$)
(c) lying dorsally 20° [illegible] plane ($Rn_{20°}$)
(d) lying horizontally (Rn_{0})

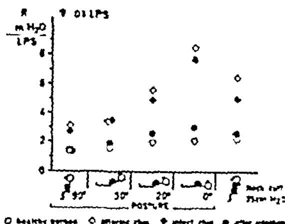


Fig. 2. The mean values of nasal resistance (cm H_2O/LPS at $\dot{V}$ = 0.5 LPS) in relation to different postures and during neck vein compression in four different groups of subjects.

(e) sitting erect, the neck veins compressed by 35 cm H_2O by an inflated cuff (Rn_c)
(f) sitting erect making prolonged inspirations and expirations 5 second each with respiratory pressures (I a) of 20 cm H_2O positive and negative. The change of Rn the mean value during three consecutive inspirations is called ΔRn.

Rn was measured 2 minutes after the subject had taken up a new position or after 2 minutes neck vein compression, respectively. After each measurement the subject was returned to the sitting position for 2 minutes.

RESULTS

Fig. 2 shows the mean values of Rn in different postures and during neck vein compression. Table 1 also shows the effect of prolonged inspirations. The results make it possible to divide the material in two parts, one consisting of the healthy individuals, groups 1 and 4, and the other part of the rhinitic patients, groups 2 and 3. In the sitting position Rn was 1.4 cm H_2O/LPS in the two healthy groups at the flow rate used. The corresponding values for the rhinitic patients were twice as high. Rn increased in the 30° position only to a small degree in all subjects. $Rn_{20°}$ in group 1 and 4 reached 2.0 and 2.6 cm H_2O/LPS respectively, an increase of 0.6 and 1.2 cm H_2O/LPS. In the rhinitic groups $Rn_{20°}$ became 5.6 and 4.9 cm H_2O/LPS, an increase of 2.5 and 2.2 cm H_2O/LPS, respectively, from $Rn_{90°}$.

In the horizontal position there was only a slight additional increase of nasal resistance in the healthy subjects while in the rhinitic group Rn was about three times higher than in the sitting position. The differences between $Rn_{90°}$ and $Rn_{0°}$ in groups 2 and 3 (2.9 and 2.8 cm H_2O/LPS, respectively) are statistically significant (0.05 > p > 0.025).

During compression of the neck veins with 35 cm H_2O inflation pressure Rn increased to a level between Rn_{w} and Rn_{g} in all groups. The differences of the mean values between Rn_{g} and Rn_{C}, seen in groups 2 and 3, are statistically not significant ($0.2 > p > 0.1$ and $0.1 > p > 0.05$ respectively). Changes of Rn recorded during prolonged inspirations occurred in only five out of 15 tested subjects in the two healthy groups, 1.6 cm H_2O/LPS being the maximum. The same test performed in 12 out of the rhinitic patients revealed increases of Rn in all of them, 5.0 cm H_2O/LPS being the maximal value.

DISCUSSION

Comparative measurements of the nasal airway resistance in different body positions were reported for the first time in 1964 by the present author in a series of patients with allergic rhinitis. In 17 out of 25 subjects a "positive posture reaction" was found, i.e. a considerable increase of nasal airway resistance took place in a body position 15° below the horizontal plane. This reaction was not seen in healthy individuals and could not be reproduced in the patients after successful treatment of the nasal disorder. The "positive posture reaction" was thought to be caused by mucosal congestion due to a pathological hypotonicity of the mucosal vessels, which could not resist an increase of the hydrostatic venous pressure.

Recording of this reaction was intended to provide a simple test for objective evaluation of the effect of treatment of allergic rhinitis (Rundcrantz, 1964). Solomon (1966) reported similar observations. Patients with concurrent allergic rhinitis showed significantly greater decreases in nasal airway patency during recumbency than were observed in comparable control subjects.

It was logical, however, to assume that postural variations of nasal patency would occur not only in allergic rhinitis but also during a common cold. Accurate measurements might also prove the healthy nose to be affected by body position.

Although the rhinomanometric measuring method used in 1964 gave good information on nasal resistance it did not fulfill strict physiological demands. A new improved method for measuring nasal airway resistance was therefore used in the present investigation (Ingelstedt *et al.* 1969). This method has proved to give accurate and reliable values of Rn of the entire nose during spontaneous respiration without intranasal instrumentation. It is the only method described where the effects of high transmural pressures on the nasal mucosa can be studied. With this method the mean nasal airway resistance was previously determined in healthy individuals, 1.39 cm H_2O/LPS at a flow rate of 0.5 LPS. No difference between inspiratory and expiratory resistance was found. In the present investigation almost the same mean value was found in healthy persons in the vertical position (1.4 cm H_2O/LPS) and also—as could be expected—in patients reexamined after

recovery from common colds In the horizontal position and also at an elevation of 20 there was actually an increase of Rn in these healthy subjects, although the mean value did not surpass 2.1 and 3.0 cm H_2O/LPS in groups 1 and 4 respectively The increase of Rn in the horizontal position was not experienced by the subjects in these groups.

In elevations of 20 or less the postural effects on Rn in the rhinitic patients resulted in a considerable decrease of nasal patency While in positions above 20 the ratio of Rn between the healthy persons (group 1) and the rhinitic patients (groups 2 and 3) was about 1 2 it became about 1 4 in the horizontal position Most of the rhinitic patients experienced nasal blockage in the horizontal position The mean values of Rn in this position (7.7 and 8.5 cm H_2O/LPS) are more than twice as high as the normal resistance of the total airways at the same flow rate (Butler 1960) Four of the rhinitic patients developed Rn in the horizontal position surpassing 12 cm H_2O/LPS, the maximum was 21 cm H_2O/LPS. These patients had to make respiratory efforts exceeding 5 cm H_2O positive and negative in order to produce a V of 0.5 LPS

As shown by Sternberg (1925) compression of the neck veins causes swelling of the nasal mucosa in experimental animals as well as in man. v Dishoeck (1938) devised a technique for diagnosing venous sinus thrombophlebitis by utilizing this phenomenon The vessels of the nasal mucosa mainly open into the internal jugular veins. An increase of the pressure within these veins is transmitted to the nasal vessels as there are no valves in the venous system of the head and neck. A filling or distention of the cavernous tissue results in mucosal congestion In the erect position the pressure within the jugular vein is almost zero, increases very little down to the 30 position, but from the 20 down to the 0 position increases continuously to 4.5–11 mm Hg (6–15 cm H_2O) (Jonson & Rundcrantz, 1969) The same venous pressure can be reproduced by compressing the neck veins by 35 cm H_2O by an inflatable cuff

The results of earlier authors, showing that the recumbent position causes a swelling of the nasal mucosa in certain conditions owing to an increase of the hydrostatic venous pressure could thus be confirmed quantitatively by the present investigation During compression of the neck veins in the erect position the nasal airway resistance increased in all groups to a degree identical with that in the position between 20 and 0 The recording of Rn was performed after only 2 minutes in every new situation in order to study the vascular reactions exclusively and to avoid any possible development of edema

The effects of prolonged respiration with high transmural pressure on the nasal mucosa was lately described by the present author (Ingelstedt et al 1969) and was further studied in the present investigation When a constant negative pressure of 20 cm H_2O prevailed within the nose during an inspiration for 4–6 seconds, very slight effects on Rn or none at all were seen in healthy persons. In rhinitic patients the same manoeuvre resulted

in a continuous increase of Rn, sometimes to a high degree. During the following expiration with the equivalent positive intranasal pressure a corresponding decrease in Rn occurred but very rapidly at the beginning of the expiration. It is therefore easier to measure ΔRn during inspiration.

The mucosal reactions to high transmural pressures in rhinitic patients are supposed to be caused by a filling and an emptying of the vessels due to the same vascular hypotonicity that may be responsible for the postural swelling. This theory is supported by the fact that the positional effects on the mucosa seem to be almost eliminated after successful treatment of the allergic rhinitis and also after recovery from nasal infection. Another support is that the transmural pressure effects disappeared after administrations of ephedrine nose drops.

Nothing is known with certainty about the reasons for the change of vascular tonus in the nasal mucosa which is supposed to take place during allergic or infectious rhinitis possibly vegetative dystony in allergy (Kuntz, 1945 1950 Williams, 1951) or some viral or bacteriological noxae to the mucosal vessels during infectious rhinitis may be relevant. Although decrease of nasal patency in the recumbent position was reported as early as the 19th century the biological consequences of this phenomenon during disease have not been investigated and only a few authors have discussed the problem whether it may be instrumental in propagating disease to the paranasal sinuses, to the middle ears and also to the lower respiratory pathways (Aaneland, 1964 Ingelstedt *et al* 1967 Rundcrantz, 1969)

During bed rest nasal obstruction in rhinitic patients is probably much more extensive than that seen in the present investigation after only 2 minutes in the recumbent position It is, however not only an uncomfortable condition which interferes with sleep and often forces the patient to breathe orally Presumably positional congestion of the nasal mucosa also blocks the ostia of the paranasal sinuses which would be analogous to what was found in the Eustachian tube during infection (Rundcrantz, 1969) Zange (1940) pointed to the fact that the submucosal cavernous tissue of the nose is not limited to the inferior and middle turbinals and the septum but is also located in the areas of the ostia, which would be the anatomical basis for great variations of swelling

Examinations on patients with acute rhinitis and maxillary sinusitis in the sitting position have revealed a high incidence of impaired permeability of the maxillary ostium as shown by Drettner (1965) and Drettner & Lindholm (1967)

Thus it is important in certain conditions to prevent or alleviate the effects of the recumbent position on nasal patency The current medical advice to go to bed in order to cure a common cold may be of doubtful value. It may be better for the patient to sit upright But when the rhinitic patient goes to bed at night or if he chooses to stay in bed for some days he should take nose drops and other decongestive agents and also take up a position with the upper part of his body well above the critical angle of 20

recovery from common colds. In the horizontal position and also at an elevation of 20° there was actually an increase of Rn in these healthy subjects, although the mean value did not surpass 2.1 and 3.0 cm H_2O/LPS in groups 1 and 4 respectively. The increase of Rn in the horizontal position was not experienced by the subjects in these groups.

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CRITICAL REVIEW OF NECK DISSECTIONS

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It is a fact that only one per cent of patients with the cancer of the head and neck die from the distant metastases. For that reason great hope has been laid on neck dissection. The results so far show that after neck dissection 30–35 per cent of the patients remain alive. According to their personal observation the authors tried to explain this problem.

In 1906 in America Crile published his work "Excision of Cancer of the Head and Neck" in which he published the enormous total of 4500 cases of the cancer of the head and neck, deceased because of the uncured primary tumour or the metastases of the neck, and only 1% because of distant metastases. In his series, although small he proved that the patient with cancer of the head or neck in addition to excision of the primary lesion, had a 25 per cent better chance of living for three years without disease than one treated surgically for the primary lesion only.

In 1963 Gušić & Krajina on the basis of their statistics of 168 cases of neck dissections had 35% of cured cases after neck dissection, either in the first instance with the excision of the primary tumour or subsequently at the appearance of metastasis. However in studying our cases over as many as 15 years we became convinced by Crile's statement that patients rarely die of distant metastases, but that there occur also relapses of the metastases after neck dissection (especially with cancer of the pharynx) and that surgically in such cases we are unable to overcome the malign lesion.

We have made it our task to elucidate this fact and to find out whether we could modify the extent of our procedure in order to minimize the number of relapses. In the first place we were interested to know whether neck dissection removes all the lymphatic tissue and whether the lymphatic tissue will regenerate after such an operation. In their experiments on rabbits and their observations on patients by means of lymphography Sigel & Fisch (1965) showed that after neck dissection, lymph vessels can flow away through subcutaneous and deep lymph system ipsilateral or contralateral superficial and deep system. These observations on animals were registered after only four weeks, and on human beings up to one year postoperatively. Welsh & Welsh (1963) and Pressman *et al* (1960) pointed out that after neck dissection contralateral flow usually appears. In 1947 Furuta showed in rabbits that regeneration of the lymph node appears. He stressed the animal's youth as being very important for regeneration. Lympho-reticular



Fig 1 Lymphoreticula element f f t tissue f the neck.

elements were found in the blocked cervical region after removal of a lymphatic segment as early as four weeks after surgery Sanders & Florey state that after neck dissection regeneration of the lymphatic tissue in the region of the neck cannot appear except as a compensatory increase of the remaining lymph nodes.

Normally the lymph glands of the neck vary in size from 1 mm to 1.5–2 cm These bigger nodes are found in the submandibular and subdigastric region Our hist logic examinations of the fat tissue in the lower part of the neck (30 cases) which lies laterally from the jugular vein shows, in 90% of cases, smaller or larger accumulations of lymphatic tissue beginning from the smaller group of lymphoreticular elements up to the real lymph node formed (Figs 1 and 2) This means that this is the reservoir and the poten tial probability for the formation of new lymph nodes after neck dissection Lymphatic tracts of the uperficial nd deep system of the neck also show such lymphatic cc umul tions, which in the case of a neck dissection may form real new nodes meta t ses

At present we n t abl t define by the histological structure the biological chara t f th extent of the tum ur's malignity Up t the



Fig 2 Lymph gland f f t tl f the neck.

present time we stated that the immaturity of the tumour goes parallell with the greater malignity However the majority of our cases in which we observed such relapses after a neck dissection were histologically mature tumours, even with a very pronounced cornification (Fig 3) *This led us to* doubt the statement that only histologically undifferentiated structure designates a greater degree of tumour malignity Moreover we had a number of cases of undifferentiated tumour of the epipharynx which had no metastases and which remained without relapse for several years.

The next question posed is where does the greater malignity of a tumour show itself?

According to our clinical observations we think that this malignity manifests itself in a rapid spreading of the tumour into the lymph system of the neck, and that not only macrometastases, in the sense of the formed nodes, but also micrometastases in smaller lymphatic accumulations are formed By histological examination of the fat tissue of the lower part of the neck we found in 20 cases two with such micrometastases, which could have escaped our notice by classical neck dissection (Fig 4) Further characteristic of the

Fig 3 Cancer with the complicati n.

malignity besides these multilocular metastases of the neck is the spreading of the tumour beyond the limits of these glands into the surrounding fat and fibrous tissue and muscles The organism fights against an invasion by the formation of scar tissue or the formation of so-called mesenchymal sheets which may go along the great blood-vessels or nerves, in places where, normally there is more fibrous tissue in the shape of fascia or loose connec tive tissue Histological analysis of such fibrous sheets has shown malign cells which it is true are scarcer but are strewn about along these structures and show possibility of further growth and spreading (Fig 5) Moreover by histological analysis of the connective tissue and the fascia around the carotid artery we have found smalle or larger accumulations of the lymph tissue which can also be affected by such spreading of the tumour

The metastases of the neck do not mean only the anatomic spreading of cancer into the first lymph glands but, first of all the decrease of the balance in the struggle of the organism against carcinoma Accordingly if the appearance of m tastases alone signifies a further degree of the fragility of the rganism in relation to the cancer then in cases of stronger malign lesion, such multiple metastases and the sp eading beyond the glands into the surrounding structures of the neck will lmost be a rule In this way we



Fig. 4. Micrometastasi i the lymphati tissue of th neck.

can understand the appearance of metastases proximally and distally after neck dissection because of the presence of lymphatic elements in these regions. Besides, such relapses can take place along the carotid artery as *growth of the remaining malign structures either in the fibrous tissue or* in the lymphoreticular structures. Finally such relapses may appear also in the superficial lymphatic structures of the so-called subcutaneous lymphatic region or the lymphoreticular elements.

Such isolated relapses in time become increasingly diffuse in character because of the malignity of the tumour not only on one side of the neck, but bilaterally when they lead to complications either on the part of the great vessels or other important structures of the neck

From clinical observation we were convinced that such relapses are more frequent on the side where there is primary lesion and the first metastases, even before they appear contralaterally which, according to the flow of lymph should come previously However their appearance on the same side first speaks for the fact that after neck dissection there remained either micrometastases or various isolated structures in the fibrous plate or *lymphoreticular accumulations.*

On the basis of these observations we believe that from the surgical point of view we have to remove with the neck dissection the whole fat tissue subcutaneous region, and especially such larger accumulations which lie like a conus in the lateral distal part of the neck and cranially in the depth



Fig 5 Groups of malign cells in the fibrous tissue of the neck.

behind the angulus mandibulae Fibrous plates or structures should be removed *in toto* and not be considered as the borderline of the malign process. Finally in each operation such structures must be immediately examined histologically so as to be maximally radical and possibly postoperatively apply roentgen therapy or other anticancerous means.

All these remarks refer only to surgical possibilities of the removal of a malign tumour without going into the essence of the biology of this malign growth. In this way our aim would be to increase if possible the percentage cure after neck dissection

ZUSAMMENFASSUNG

Es ist eine Tatsache, dass nur 1% von Patienten mit malignen Kopf und Halstumoren an entfernten Metastasen sterben Aus diesem Grund setzte man grosse Hoffnung auf die radikale Halsreaktion. Die bisherigen Resultate zeigen, dass nach dieser Operation 30–35% von Patienten am Leben bleiben. Die Autoren versuchen dieses Problem nach eigenen Erfahrungen zu erklären.

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ON EXTRACRANIAL MENINGIOMA

Case of primary meningioma of nasal cavity

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A case of primary extracranial, ectopic meningioma of the right nasal cavity in a 40-year-old woman is described. There were no clinical or roentgenological signs of intracranial origin. The tumour had probably risen from ectopic arachnoidal cells in the posterior upper part of the nasal cavity. Published cases are exemplified and a systematic classification of extracranial meningiomas is suggested.

Apart from intraorbital meningiomas, extracranial and particularly primary extracranial or ectopic meningiomas are extremely rare. It was therefore thought legitimate to report the following case of probably primary ectopic meningioma situated in the nasal cavity and not demonstrably related to any intracranial lesion.

Case report

The patient was a 40-year-old married woman who had previously always felt well except for an episode of eczematous changes in the auditory ducts in 1963. Routine examination on that occasion had revealed mild deviation of the nasal septum but otherwise nothing remarkable. The patient returned to the ENT Department in October 1967 because of a feeling of a lump in the throat that had proved refractory to penicillin. Routine examination now revealed in the right side of the nasal cavity a small bullous lump which was thought to be a polyp. The blood picture, differential count and the ESR (7 mm/1 h) were normal.

In November 1967 the polyp was resected. The growth was hard and of unusual appearance. Part of the specimen was therefore sent to the department of bacteriology and part to the department of pathology. Culture gave no growth of pathogenic bacteria, but microscopical examination of the specimen, which was perhaps not quite representative, revealed a picture of what was called an angioleiomyomatous polyp without signs of malignancy.

A more representative specimen was obtained in January 1968, when the histological picture with scattered psammoma bodies was compatible with a diagnosis of meningioma of meningotheliomatous type.

The following month the patient was admitted to hospital for investigation

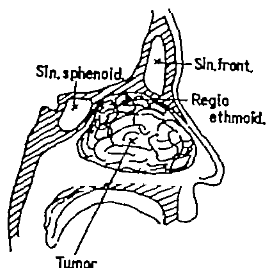


Fig 1 Diagram showi g lz od site f tum or

of the origin and spread of the tumour No neurological or mental disorders were demonstrable The total protein content of the CSF was normal (38 mg/100 ml) Ophthalmological and ophthalmoneurological investigation revealed nothing remarkable Skull X ray tomography and bilateral carotid angiography showed no abnormalities. No signs of an intracranial expanding tumour could be demonstrated. But right external carotid angiography and tomography revealed a tumour of the right nasal cavity After the site and the extent of the tumour had been demonstrated the right external carotid artery was ligated and a tumour occupying the major part of the right nasal

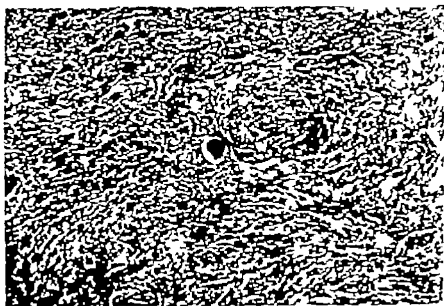


Fig 2 Microphotograph of meningioma with psammoma bod middle of reproduction.



Fig 3 Fro tal X-ray projection howing t mour I right naso-ma illary region.

cavity (Fig 1) was surgically removed *ad modum Dencker* via the right maxillary sinus and inferi r right conchotomy The tumour which bled readily apparently originated from the ethmoidal region, where it had eroded part of the ethmoidal cells. No direct connection with the lamina cribriformis could be demonstrated. After the tumour had been removed the upper nasal region was readily accessible and ethmoidectomy was done. The histological picture of the tumour was compatible with a diagnosis of meningioma of mainly meningotheliomatou type with a few psammoma bodies (Fig 2)

After the operation the mucosa in the operative cavity healed and no signs of a recurrence have hitherto been observed.

DISCUSSION

Meningi ma is u ually situated intracranially and constitutes about 15% of all intracranial tumours. M ningi m s are built up of arachnoidal fibroblasts and hi tologi ally three main types are distinguished Meningiotheliomatous, psammomatous (wit abund ce f psammoma bodies) and fibro-

blastic (Kernohan & Sayre 1952) As a rule, meningiomas are benign. Only few malignant meningiomas with a tendency to metastasise have been reported (see below)

Formerly tumours containing psammoma bodies were not infrequently called "psammomas"

One group of tumours with a certain predilection for the nasal cavity and its sinuses have been described in less recent German literature as endotheliomas (Schmidtmann 1928) These tumours may have originated from endothelium, but judging from their histological descriptions, some of them may have been very vascular meningiomas. Owing to differences in nomenclature it is sometimes difficult to decide whether certain tumours described in the literature should be regarded as examples of meningioma or not Thus, in 1949 Gögl reported on a group of tumours of the nose or nasal sinus, which he called psammo-osteoid fibromas. Some of those tumours may have been meningiomas.

Extracranial growth of meningiomas is rare Judging from published cases, four types of such growth can be distinguished.

1 Primary intracranial meningioma
   A With growth by continuity through the cranium and out into surrounding tissues.
   B Malignant with extracranial metastases.
2. Primary extracranial meningioma
   A Arising from the arachnoidal membrane in the vicinity of foramina in the cranium (or vertebral column) (i) Orbital (ii) non-orbital
   B Without direct connection with cranial foramina, true ectopic meningiomas. (i) Cutaneous (ii) non-cutaneous

Primary intracranial meningiomas with growth by continuity into the surrounding structures constitutes the majority of cases with extracranial growth. Such cases have been described by Belal, 1955 Faulwetter 1959 and Rosalki & McGee 1962, and others.

The literature also contains reports of intracranial meningiomas with metastases in the lungs (Jurow 1944 Christensen *et al* 1949) and in the mediastinal lymph nodes (Vlachos & Prose, 1958) These reports also contain references of publications of cases with metastases in, *inter alia,* the liver

Primary extracranial meningioma originating from foramina in the cranium probably arise from arachnoidal fibroblasts in or near the nerve sheaths. *To this group belong many of the intraorbital meningiomas* arising from the foramen opticum. Such cases have been described by Craig & Gogela (1941) and others Heye *et al* (1960) described a meningioma arising from the region of the foramen lacerum.

Extracranial meningiomas unrelated to foramina in the cranium, i e true ectopic meningiomas, and situated in the maxillary sinus have been reported by Shahen (1931) and Hill (1962) and others. In 1952 Zacharlae published a case of meningioma of the temporal bone without demonstrable intra

cranial growth Pendergrass & Hope (1953) described a case of meningioma over the left frontal bone. These tumours had probably arisen from embryonal ectopic arachnoidal cells. This probably holds also for the cutaneous meningiomas surveyed in 1956 by Bain & Shnitka. Most of these tumours were confined to the head or the skin near the vertebral column.

In our case there were no clinical or roentgenological signs of intra cranial meningioma. Neither were any direct connections with nerve struc tures demonstrable. The growth thus appeared to be a primary extracranial ectopic meningioma. Its position suggested that it had arisen in the upper dorsal part of the nasal cavity in the posterior ethmoidal region without involving the lamina cribriformis. The tumour had probably originated from ectopic arachnoidal cells in the posterior ethmoidal region

Meningioma of the nasal cavity or sinuses is very rare, particularly pri mary extracranial, ectopic meningioma. Thus, the register of the Armed Forces Institute of Pathology (Ash *et al.*, 1964) contains only seven cases of meningioma of the nasal region, six of which were however combined with intracranial meningioma. The seventh case was regarded as having arisen extracranially in the maxillary sinus. Cushing & Eisenhardt (1938) reported no case of primary extracranial, ectopic meningioma in their large textbook on meningiomas.

In a recent survey of primary extracranial meningiomas, excluding intra orbital meningiomas, Suzuki *et al* (1967) traced 13 published cases, seven of which were cutaneous and situated mainly in the head or in the region of the skin along the spine. Of the remaining six non-cutaneous meningiomas, one was reported by Zachariae (1952) and one by Pendergrass & Hope (1953) They also referred to four cases described by New & Devine (1947) one of which was localised to the nasal region and three to the frontal sinus, but in one of the cases the tumour had encroached upon the antrum and ethmoidal sinus as well as the medial orbital wall To these cases Suzuki *et al.* (1967) added a personal one in which the tumour had originated in the pterygo-maxillary fossa.

ZUSAMMENFASSUNG

Es wird der Fall ei es M ningeom in der Nasenhöhle einer 40-jährigen Patientin beschrieben. Weder klinisch noch röntgenologisch konnte der Nachweis erbracht werden, dass der Tumor i trakra i llen Ursprungs war Das Gewächs war wahr scheinlich aus ektopischen Arach oidzellen im dorsalen Teil der Nasenhöhle ent standen. Eine Klassifikation extracranieller Meningeome sowie Beispi le ander weitig beschriebener Fälle wird gegeben.

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A SWALLOWING DISORDER DENOTED IN TARDIVE DYSKINESIA PATIENTS

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An bnormal lingual conditi n has been detected in three linically diagnosed Tardive Dyskinesia patients. Cinefluorographic analysis was the technique utilized to study this condition and a frame by frame trac ing showed pronounced lingual thrust to be present in each case.

Tardive Dyskinesia, an extrapyramidal syndrome caused by long term ingestion of phenothiazine drugs, is characterized by choreathetoid movements, tonic and clonic posturing, and involuntary buccolingual movements. The buccolingual movements may be so severe that the patient has difficulty talking and swallowing. Studies to investigate the swallowing disorders of extrapyramidal origin have been limited.

In the present investigation the swallowing pattern for three patients who had clinically been diagnosed as having Tardive Dyskinesia were studied by the use of cinefluorographic analysis. A Philip s cinefluorographic unit with a 16 mm Arriflex camera was used in this research. A Kodak Cine-X projec tor permitted a frame by frame analysis of the swallowing cycle. The results for all three pat ents were the same The type of pattern denoted in the swallowing cycle was that of a severe tongue thrust. Fig 1 illustrates this thrusting condition. Frame 1 in Fig 1 shows the tongue being protruded between the front teeth as the swallowing cycle begins. In frames 3, 4 5 6, 7 8 and 9 the tongue begins to move backward in the oral cavity In frame 10 the tongue begins again to move forward instead of completing its upward and backward m vement and the Rugar bolus which the patient was attempting to swallow never progresses into the pharyngeal area during the first cycle After numerous attempts and after many more tongue thrusts the patient was able to move the bolus backward into the oral cavity

One of the prim ry difficulties in swallowing for the Tardive Dyskinesia patient has been the consi tent tongue thrust. Instead of the tongue moving upward and backward, such as in the normal swallowing pattern, a jutting

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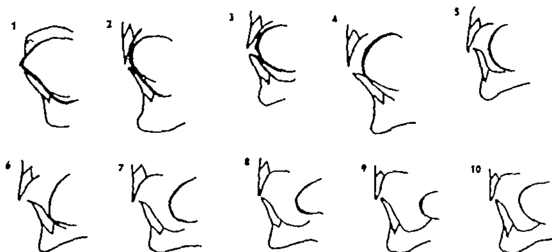


Fig 1 Cl efl orographic tracing f th t ngue thrusting condition in a Tardi e Dyskin-esl patient

forward motion has been detected from the cinefluorographic analysis. It would appear that any alleviation of this thrusting condition would help these patients develop a more normal swallowing pattern

ZUSAMMENFASSUNG

Eine Zungenabnormalitat wurde bei drei Patienten gefunden, bei denen die klinische Diagnose tardive Dyskinesie (spät auftretende Dyskinesie) gestellt wor den war Cinefluorographische Analyse wurde beim Studium dieser Störung verwendet. Die Bilder zeigten, dass in jedem Falle ein ausgesprochener Zungenstoss bestand.

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THE ROTATORY NYSTAGMUS RESPONSE IN CHILDREN

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The nystagmus pattern obtained by a standardized vestibular stimulation was studied in children from birth to 15 years f age. The speed of the slow compone t decreased with increasing age. The differences in the speed between the age groups were most pronounced at the beginning of the vestibular test, whereafter they dimi ished successively The duration of the nystagmus was shortest in hildren aged less than 12 months. The nystagmus amplitude and the speed of the fast component decreased with increasing age the differences between the age group at the beginning were th same as at the end of the vestibular response. The nystagmus intensity was classified in two parts on depending on the peripheral, vestibular activity and the other on the central activity According to this classification, the small children in relation to the older ones had high peripheral and a low central nystagmus intensity

In newborn children, the configuration, dimension and position of the bony labyrinth are almost the same as in adults (Sato, 1903) The three semicircular canals of the newborn react to rotatory as well as to caloric stimuli, according to Galebsky (1927) who found a pronounced post-accel-eratory deviation of the eyes in the direction of the slow component but an almost complete lack of the fast components. In adults, the sum of the nystagmus amplitudes during a stated time decreases with increasing age (Minnigerode & Grohmann, 1966) However little is known about the nystagmu pattern during the period of growth. The aim of the present investigation was therefore to study the peripherally induced slow phase and the centrally induced fast phase in the nystagmus response in children This was done by analysing the DC-recorded per rotatory nystagmus pattern obtained by a standardized angular acceleration in darkness.

MATERIAL AND METHODS

The material consisted of 84 children (newborn to 15 years of age) divided into six groups according to age one hour to 5 days ($n = 12$) 3–12 months ($n = 10$) 1–3 years ($n = 10$) 4–6 years ($n = 13$) 7–9 years ($n = 16$) and 10–15 years ($n = 23$) The newborns were taken from the delivery department,

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and the rest were children hospitalized at the paediatric and otologic departments for minor disorders. No central depressant drug was allowed for the preceding 24 hours

During the vestibular test, the children sat in a rotation chair either alone or on the knees of an assistant Their heads were fixed with the lateral semicircular canals oriented in the horizontal plane After calibration of the horizontal eye movements for 60 degrees around the midpoint the test was performed in total darkness. The calibration was not performed in the 12 newborn they are incapable of ocular fixation The children more than 3 years of age were instructed to keep their eyes open and to report on perception of rotation and non rotation. The angular acceleration stimulus was 120 /sec/1.8 sec. After 1.8 sec, the velocity was constant. The rotation test lasted for one minute

The horizontal eye-movements were recorded by means of electrodes applied to the anterior part of the temples at the eye level. This permitted a DC-recording technique (Lundgren *et al.*, 1969) The speed of the rotation chair was recorded by means of an optical device. The signals were recorded on a 4-channel ink writer (Mingograf 81 Elema Schönander Sweden) fed with a paper speed of 2.5 cm/sec By use of an infinite time constant and an upper limit frequency of 15 Hz, the rise time was 0 023 sec.

From the per rotatory recordings, we analysed the following variables the speed of the slow and the fast components at 2, 4 10 18, and 30 sec after the start of the rotation and the amplitudes at the same points, the nystagmus frequency per 10 sec of rotation (Tibbling & Henriksson, 1968) and the duration of the per-rotatory nystagmus. The data were statistically evaluated by Student s *t* test and Spearman s coefficient of rank correlation (Documenta Geigy 1962)

RESULTS

Ten in the newborn group had no nystagmus beats during the rotation. At one to two seconds after the start of rotation, the vestibular reactivity of the subjects appeared as a deviation of the eyes in the direction of the slow component. The duration of the deviation averaged 13 ± 0.8 sec ($\pm$ S.E.M.) The mean size of the deviation was estimated at 15 degrees. The other two in the newborn group had irregular bursts of nystagmus lasting a few seconds. The data of the newborn group are excluded from the following results.

Speed of slow component

Table 1 records the mean speed of the slow components with standard error of the mean and the ranges for the age groups. The speed of the slow component decreased with increasing age The difference in the speed between the age groups was most pronounced at maximum of stimulation. The mean eye speed 2 sec after the start of rotation was significantly higher

TABLE 1 *Speed of slow component at different times after the acceleration of 120 °/sec/1.8 sec for the five age groups studied*

The results are given as mean ± S.E.M. (ranges) degrees/sec. The asterisks refer to the significance limits of $p < 0.05$ ( ), 0.01 ( ) and 0.001 ( ). The statistical evaluation has been performed with the 10–15 year age group as reference group.

	Time after start of rotation (seconds)				
Age group	2	4	10	18	30
3–12 months	127 ± 10	100 ± 10	49 ± 4	19 ± 3	—
= 10	(86–168)	(74–160)	(32–66)	(4–39)	
				n = 9	
1–3 years	100 ± 4	81 ± 8	47 ± 5	32 ± 6	21 ± 3
= 10	(80–114)	(57–137)	(28–80)	(11–50)	(11–29)
				n = 8	n = 6
4–6 years	92 ± 6	6 ± 6	53 ± 5	30 ± 3	14 ± 3
13	(48–126)	(32–115)	(22–78)	(13–41)	(6–33)
7–9 years	71 ± 6	67 ± 4	41 ± 3	23 ± 3	12 ± 2
= 16	(26–104)	(22–92)	(16–63)	(12–48)	(4–27)
10–15 years	67 ± 4	50 ± 4	32 ± 3	19 ± 2	11 ± 2
23	(24–93)	(23–80)	(12–66)	(1–43)	(1–29)
					n = 19

for the children 3–12 months old (127 °/sec) than for the 10–15 year group (66 °/sec, $p < 0.001$). Even between the age groups 7–9 and 10–15 years, there was a highly significant difference in the speed at the fourth and tenth second.

Duration

Table 2 records the duration of the per-rotatory nystagmus. The 3–12 month group had a lower duration of the nystagmus (21 sec) than the older

TABLE 2 *Duration of the per rotatory nystagmus and nystagmus frequency after an acceleration of 120 °/sec/1.8 sec for the different age groups.*

The results are given as mean ± S.E.M. (ranges). For explanation of the asterisks, see Table 1

		Frequency (beats/10 sec)	
Age group	Duration of nystagmus (sec)	0–10 sec	10–20 sec
3–12 months	21 ± 1	15 ± 1	10 ± 1
= 10	(17–32)	(10–22)	(1–16)
1–3 years	33 ± 4	20 1	11 ± 2
= 10	(19–52)	(16–26)	(7–19)
4–6 years	38 ± 2	21 2	15 ± 1
= 13	30–55)	(13–29)	(7–25)
7–9 years	41 ± 2	25 2	17 ± 1
16	(25–65	(9–12)	(6–30)
10–15 years	[illegible]	2 1	15 ± 1
23		[illegible] 41)	(27)

TABLE 3 *Nystagmus amplitude at different times after the acceleration of 120 /sec/1.8 sec for the five age groups studied*

The results are given as mean ± S.E.M. (ranges) degrees. For explanation of the asterisks, see Table 1

Age group	Time after start of rotation (seconds) 2	4	10	18	30
3–12 months	45 ± 5	44 ± 3	40 ± 5	29 ± 4	—
n = 10	(25–72)	(31–62)	(20–61)	(14–50) n = 9	
1 3 years	31 ± 3	27 ± 3	23 ± 2	23 ± 4	27 ± 4
n = 10	(18–31)	(11–38)	(13–34)	(8–33) n = 8	(10–37) n = 6
4–6 years	29 ± 3	30 ± 4	30 ± 4	22 ± 3	23 ± 4
n = 13	(10–49)	(7–60)	(11 74)	(6–41)	(6–42)
7–9 years	24 ± 4	23 ± 3	20 ± 3	18 ± 3	17 ± 3
n = 16	(7–62)	(8–50)	(7–48)	(4–40)	(5–50)
10–15 years	18 ± 2	15 ± 2	16 ± 2	14 ± 2	13 ± 2
n = 23	(6–47)	(5–44)	(6–32)	(5–44)	(5–34) n = 19

children (37 sec, $p < 0.001$) The duration of the nystagmus did not differ significantly between the groups more than 12 months old

Amplitude

Table 3 records the mean nystagmus amplitude with the S.E.M and the ranges. A decrease of the mean nystagmus amplitude with increasing age was found at each time studied after the start of rotation. The average amplitude in the youngest age group 2 sec after the start was 45 degrees, which was more than twice that of the oldest age group (18) Some children in each age group had an almost constant amplitude from the beginning to the end of the vestibular response At the 30th second after the start of rotation, statistically significant differences in the mean amplitudes were still found between the oldest age group and those groups with children less than 7 years of age

Frequency

The mean nystagmus frequency per first 10 sec rose with increasing age from 15 to about 23 beats (Table 2) There was a highly significant difference between the youngest and the oldest age group Similar r lations existed during the 10–20 sec period after the start of rotation.

Speed of fast component

The mean speed of the fast components fell with increasing age this was apparent at each time studied after the start of rotation (Table 4 and Fig 1) Two seconds after the start, the mean speed was 397 /sec in children less than one year of age compared with 230 /sec in children aged 10–15 years.

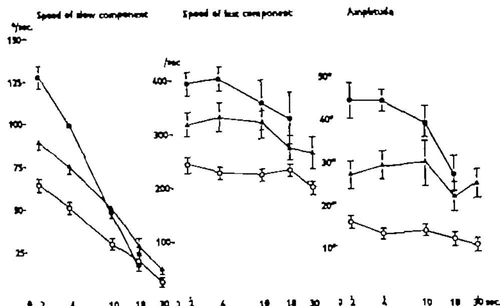


Fig 1 Per rotatory mean speed f f st nd l w ystagmus component and mean amplitudes are given with s.e.m. long logarithmic time a is. Filled circl represents mean alues t 3–12 months, tria gle t 4–6 years, nd open circl t 10–15 years.

Table 4 *Speed of fast component at different times after the acceleration of 120°/sec/1.8 sec for the five age groups studied.*

The results are given as mean ± s.e.m. (ranges) degrees/sec. For explanation of the asterisks, see Table 1

Age group	Time after start of rotation (seconds) 2	4	10	18	30
3–12 months	397 ± 20	414 ± 23	381 ± 40	308 ± 49	—
10	(320–490)	(312–618)	(162 700)	(213–616)	
				9	
1 3 years	323 ± 28	331 ± 31	277 ± 18	285 ± 22	256 ± 31
10	(230–493)	(171 504)	(175–378)	(175–350)	(154 376)
				8	6
4–6 years	321 ± 21	331 ± 28	325 ± 23	273 ± 21	268 ± 27
13	(200–450)	(220 592)	(190 461)	(182–481)	(120–420)
7–9 years	290 ± 13	310 ± 16	303 22	271 ± 19	272 ± 21
16	(206–370)	(102 420)	(180 476)	(154 420)	(120–480)
					16
10–15 years	250 ± 14	233 10	225 ± 11	235 ± 10	205 ± 14
23	(142 390)	(132 310)	(132 350)	(160–320)	(110–310)
					19

TABLE 5 *Spearman's rank correlation coefficient obtained between amplitude and speed of slow component (A) and between amplitude and speed of fast component (B) for each time studied after the start of rotation and for each age group*

Significance limits: $p < 0.05$ ( ), $p < 0.01$ ( ), and $p < 0.001$ ( ).

Time after start of rotation	Age group 3–12 months $n = 10$	1–3 years $n = 10$	4–6 years $n = 13$	7–9 years $n = 16$	10–15 years $n = 23$
A 2	0.53	−0.06	0.71	0.34	0.45
A 4	0.58	0.32	0.56	0.45	0.70
A 10	0.16	0.60	0.35	0.25	0.76
A 18	0.71 ($n = 9$)	−0.34 ($= 8$)	0.66	0.47	0.45
A 30	—	−0.39 ($n = 6$)	0.75	0.37	0.31 ($n = 19$)
B 2	0.00	−0.42	0.47	0.33	0.20
B 4	0.48	0.45	0.02	0.37	0.17
B 10	−0.09	−0.01	0.58	0.66	0.60
B 18	0.59 ($n = 9$)	0.90 ($n = 8$)	0.67	0.74	0.50
B 30	—	0.54 ($n = 6$)	0.55	0.58	0.77 ($= 19$)

In each age group the speed at the end of the vestibular test was lower than at the beginning.

Table 5 shows the results of *the correlation study* between the amplitude and the speed of the slow component and between the amplitude and the speed of the fast component, for each age group and at each time studied after the start of rotation. Between the amplitude and the speed of the slow component significant correlations were found in 8 out of the 24 correlation groups tested Four of these groups belonged to the 10–15 year age group Between the amplitude and the speed of the fast component, significant correlations were obtained with increasing age and increasing time after the start of rotation

DISCUSSION

The speed of the slow component is recognized as the best parameter for reflecting the degree of the vestibular stimulation (Henriksson, 1955) A high speed of the slow component is considered an expression for a high peripheral activity In the present study the youngest children had a very high maximum *speed of the slow component*, which indicates a vestibular response more intensive than that of the older ones. In some of the children less than one year of age, there was even an oversteering, since the eye speed was slightly higher than the speed of the rotation equipment The results agree with the findings of Rossberg (1964) and Minnigerode & Grohmann (1966) that in adults the sum-amplitude for a stated time is reduced with increasing age. Since the sum-amplitude equals the eye distance covered, the sum amplitude determined within a stated time will be an expression

for the mean speed of the slow component. The present observation—that the maximum slow phase eye speed diminished with increasing age—also agrees with the results published by Henriksson (1955) that adults have a lower eye speed than the speed of the rotating chair providing the examination is performed in complete darkness.

The duration of the nystagmus is in clinical practice often used as a measure of the vestibular activity A strong vestibular response is expected to be associated with a long duration of the nystagmus, and *vice versa* The present results of the nystagmus duration in infancy however demonstrate that a short duration can also be combined with a high maximum speed of the slow component. It can therefore be concluded that the nystagmus duration is a poor parameter of the strength of the vestibular activity in children. This may explain why Zelenka & Slaninova (1964) reported that children more than 7 years of age had a vestibular hyporeflexia, since only the duration of the nystagmus was taken into consideration

The eye deviation in the newborns, in the direction of the slow component, agrees with the corresponding eye deviation obtained in adults with decreased alertness (Nathanson & Bergman, 1958) Furthermore the duration of the eye deviation in the newborns was approximately the same as the nystagmus duration in the children 3–12 months old. These facts indicate that the eye deviation reflects the vestibular reactivity in the newborns.

The results show that *the nystagmus amplitude* decreases with increasing age. At a certain age, however the amplitude is relatively unaffected by the decrease in the vestibular response as determined by the speed of the slow component (Fig. 1)

Since the amplitude depends on when the slow phase is interrupted by the centrally induced (McCabe, 1965) fast phase, the amplitude is considered to be a centrally evoked nystagmus parameter The results presented by Tibbling (1969) agree with this consideration, since nicotine has a selective effect on the amplitude and the speed of the fast component, whereas the speed of the slow component is unaffected. Nicotine is a central activating drug (Murphree *et al* 1967 Yamamoto & Domino, 1964) it is therefore probable that the nicotine induced decrease in the nystagmus amplitude is an expression of increased reaction velocity in the CNS on the sensory vestibular impulses. Analogously the high amplitudes observed in the young children may reflect a low central reactivity to the sensory impulses.

The speed of the fast component was found to fall with increasing age thi was apparent at the beginning as well as at the end of the vestibular response In these respects, the speed of th f t component varies in a manner similar to that of the amplitude (Fig 1) A fall in the speed, however correspond to a suppression of the central activity whereas a decrease in the amplitude, as discussed above may indicate the opposite condition—an increase in the central activity This suggests that these two nystagmus qualities are expressions of a functi nal counterbalance system in the brain

The nystagmus frequency per 10 sec decrea es by an increase in the ampli-

tude or by a reduction in the speed of the slow component. Since the speed of the slow component was highest in the small children as were the amplitudes, the low nystagmus frequencies obtained in this group must be caused by the great amplitudes. In spite of the reduction in the speed of the slow component with increasing age, the nystagmus frequency was not diminished this depended on the decrease of the amplitudes. Obviously the nystagmus frequency per 10 sec cannot be used as an index for the vestibular activity when there is a variation in the amplitude Consequently the nystagmus intensity expressed by the nystagmus frequency does not reflect the intensity of the vestibular stimulus. The nystagmus intensity may therefore be classified with regard to (1) the peripheral activity reflected by the maximum speed of the slow component, and (2) the central activity reflected by the amplitude With these criteria, the nystagmus response of the younger children in relation to the older ones was characterized by a high *peripheral and a low central nystagmus intensity*

The correlation study produced no evidence for a strong relation between the amplitude and the speed of the slow component in children less than 10 years of age This indicates that, at least in the younger age groups, the amplitude does not correspond to the degree of the vestibular activity Between the amplitude and the speed of the fast component, there was an increasing statistically significant correlation with diminishing vestibular response in the older age groups (Table 5) This supports the hypothesis that the amplitude and the speed of the fast component are two rather strongly associated central qualities although they may functionally counter act each other

ZUSAMMENFASSUNG

An Kindern, vom Neugeborenalter bis zu 15-Jährigen, wurde das Nystagmusmuster untersucht, welches durch eine standardisierte bilaterale Erregung hervor gerufen werden konnte

Die Geschwindigkeit der langsamen Phase nimmt mit zunehmenden Alter ab. Die Unterschiede zwischen den verschiedenen Altersgruppen treten für diesen Parameter besonders deutlich zu Beginn der Untersuchung hervor danach verringert sich der Unterschied. Im Vergleich zu den älteren Kindern ist die Nystagmusdauer bei Kindern unter einem Jahr kurz. Die Nystagmusamplitude und die Geschwindigkeit der schnellen Phase nehmen beide mit zunehmendem Alter ab, wobei die Unterschiede zwischen den Altersgruppen zu Beginn und am Ende der vestibulären Reaktion gleich gross sind. Die Nystagmusintensität wurde nach zwei verschiedenen Gesichtspunkten unterteilt ein Teil beruht auf der peripheren vestibulären Aktivität und ein zweiter Teil auf der zentralen Aktivität Im Hinblick auf diese Einteilung weisen die kleinen eine grosse periphere und eine kleine zentrale Nystagmusintensität im Vergleich mit den älteren auf.

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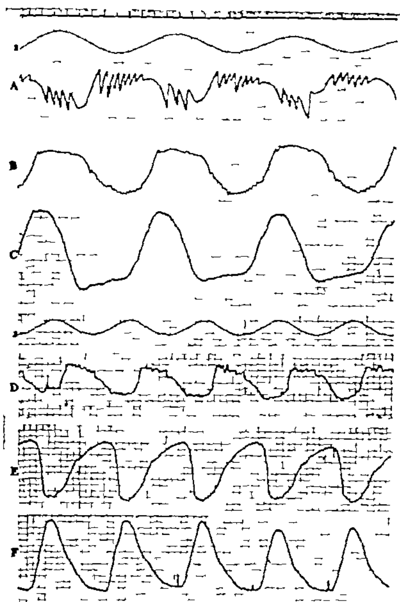


Fig 1 The effects f fenta yl l byrinth responses *1* tim in seconds *2* torsi swi g stim l ; 3 barbecu -rotati stim lus (*A*) torsion swing respo se f undrugged rabbit, (*B*) t rsi n swing recording 20 min after the i jection f fenta yl, (*C*) t rsion swi g recording 30 min after the i jection f fentanyl, (*D*) barbecu response un-drugged nimal, (*E*) barbecu response 20 min fter the fenta yl i jection, (*F*) barbecue respo se 30 min after the fentanyl injection.

tance during barbecue-rotation experiments where the quick beats are abolished.

Kompanejets (1925) studied the problem in how far direct mechanical factors can contribute to the eye deviation when the head is inclined or declined from the vertical For this purpose he examined unilaterally ophthalmoplegic patients and subjected them to various angles of lateral tilting

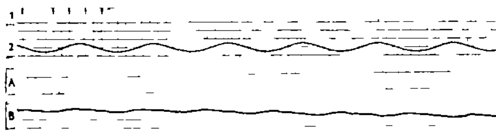


Fig 2. The effect f flaxedil on barbecue responses 1 time 1 sec (paperspeed 1 cm/sec); 2, barbecue rotation. A nd B, barbecue responses fter the i jection f fla edil. Left and right eye.

He observed that a deviation from the vertical less than 10 –60 tilting the eye deviated 4 –9 He attributed this to the fact that the centre of gravity of the eyeball lies off the rotation axis. We have some remarks on his conclusions. First his experiments range over patients with a total ophthalmoplegia. In normal subjects eye-muscle tonus could counteract the effect he found to a great extent. The centre of gravity has not necessarily the same position and does not essentially provoke the same mechanical effect.

In order to study the influence of the changes of the direction of gravity on the eyeballs themselves, the movement of the eye should be freed from labyrinthine and central influences. For this purpose we relied on a complete paralysis of the animal with flaxed l 2 mg/kg intravenously gi en

We cannulized the trachea of the rabbit and connected it to a respirator After this the animal was fastened to the rotation table whilst horizontal and vertical E.N.G. leads were taken. In this way we were able to notice whether mechanical forces provoked by the rotation about both a vertical and horizontal axis could influence movements of the eyeball. Consecutively a torsion-swing test and a barbecue test were performed The recording of the former showed no movement of the eyeballs, whilst the latter indicated minimal movements of both eyeballs 2–3 mm amplitude (1 cm = 500 microV Fig. 2)

When both movements were simultaneously tested, the pattern remained unchanged. These findings make it d ubtful whether the change in the direction of gravity in relation to the labyrinths could mechanically have influenced our recordings. Besides, in normal animals, eye-muscle tension would oppose rotation of the eyeball in its orbit under gravity influence

DISCUSSION

Dowdy *et al* (1963 and Cl ristrup & Holberg (1967) used fentanyl in combination with dr p d l The f rm used it in acute Menière s attacks with dramatic result w th n 10 min f ll wing the injecti n. They did not specify the it f on of th drug Th latter found that a caloric nystagmus wa marked! r duced n 8 f the test ubjects.

In our investigation we attempted to find out whether the cupulo-ocular and otolith-ocular reflex arches were affected alike by fentanyl. From our results it appears that the otolith-ocular reflex arch is effected the same as the cupulo-ocular arch. These findings also coincide with Alexander's law as explained by Jongkees & Philipszoon (1964). If the eye is moved into a deviated position (by a cupular or otolithic stimulation) the chances are that the central threshold for a quick phase would be exceeded.

Janssen (1962) is of the opinion that fentanyl selectively affects the formatio reticularis, the thalamus, and the hypothalamus. The fact that this drug abolishes the quick phase of nystagmus is one argument in favour of the notion that the quick phase is of central origin and not due to a simple proprioceptive reflex through the ocular muscles (Magnus & de Kleyn, 1926 Neveling & Poeck, 1960 Jongkees & Philipszoon, 1964 Kornhuber 1965).

Barbiturates and cinnarizine on the other hand, affect the cupular and otolithic responses by a general suppression of both the quick and the fast phases of nystagmus and thus reduce the compensatory eye movement of the otolithic response (Philipszoon, 1959 Jongkees & Philipszoon, 1964).

The fast phase of nystagmus can also be selectively suppressed by physiologic conditions such as sleep, unphysiologic conditions as anaesthesia, poisoning and pathologic conditions as coma and cerebellar abnormalities (de Kleyn, 1953).

The site in the case of a lesion is the formatio reticularis (Lorente de No, 1926, 1931 1933 and Teng *et al* 1958). This view corroborates the idea of Janssen that fentanyl is active in the formatio reticularis amongst others.

In the case of a selective fast phase abolition, the response to calorization would be a tonic deviation of the eye to one or other side position and would be recorded as a negative response (because of the absence of the fast phase of nystagmus). The response to a torsion swing in this case would be sinusoidal eye movement in phase with the stimulus which indicates an activity of the peripheral organs (as discussed above). This view also demonstrates the necessity of a torsion-swing test when a caloric test is negative—in order not to regard the canals as "dead" which would be a mistake. This is a case of the rare syndrome of discordance—a negative caloric test but a positive rotatory response. It is sometimes found in syphilitic vertigo (Jongkees, 1953).

ZUSAMMENFASSUNG

Die Einflüsse von Fentanyl auf die Bahnen zwischen Otolithen und Augen und auf diejenige zwischen Cupulae und Augen scheinen dieselben zu sein. Dieses Mittel unterdrückt die schnelle Phase des Nystagmus bei Kaninchen. Obwohl es die Entstehung vom Nystagmus unterdrückt, scheint periphere Aktivität de Cupulae und Otolithen unversehrt zu bleiben. Die Unterdrückung scheint also im zentralen Nervensystem stattzufinden. Hieraus ergibt sich die Notwendigkeit einer Drehprüfung auf der Torsionsschaukel als Kontrolle bei negativen kalorischen Prüfungen.

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Fig. 1 Electron micrograph demonstrates lew f Type *I* (HC1) and T pe II (HC2) hair cell from cat crista fter systemi dministration f vi myci sulf t Th vacuoli-zatio in the cyt plasm of the Type I hai cell i significant. The dendrit (*D*) is slightly swollen. The lysosom like lectron-dense granules are found t the extreme pical part of the Type II hair cell 3600.

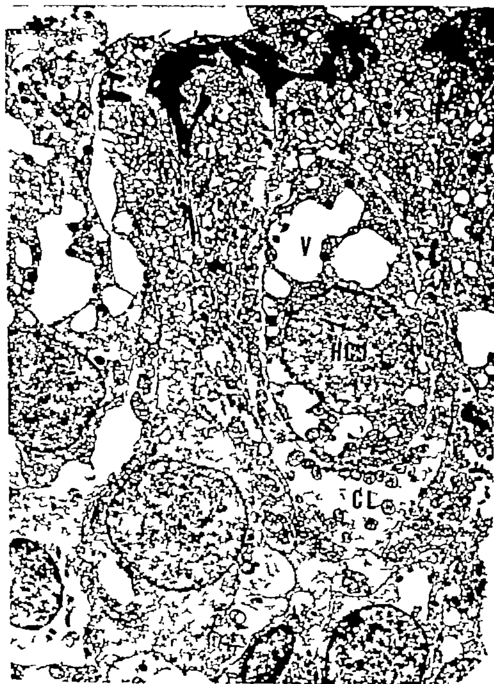


Fig. 2. Different rea of cat crista from the same imal i Fig. 1 The vacuolization i the Type I hai cell HC1 cytopl m significant. Not the shrinkage of the cytoplasmic membrane f nothe T pe I hai cell. *CL* indicates nerve chalice with swollen mitochondria nd edematou vt pl sm $100.



Fig 3. Electro m crograph exhibits view of cat crista after local lom salf l dml istrati (113 mg ml) Vesicles (V) ha f rmed ext al l l the toplasm of the Type I hal cell (HC1) Cytoplasmi protru ion int the end lymphatic space re lso present. Not the hromatin aggregati l the nucl l nd the l s of roffbell l th nerv chalices. 8400.

The entire supporting cell area was absolutely intact. The myelin sheath below the level of the basement membrane appeared morphologically intact

In the topically injected cats, the morphological alterations were much more severe than those observed in the systemically injected animals. The vacuolization of the hair cell cytoplasm was more extensive in many hair cells, and chromatin-aggregated and degenerated nuclei were regularly observed (Fig 3) Many degenerated mitochondria were found in the hair cell cytoplasm. Some cytoplasmic protrusion was noticed at the free endolymphatic surface The nerve chalices were swollen in most parts and had shrunk in some areas due to a widened extracellular space The mitochondria in the nerve chalices were slightly to moderately degenerated.

2 Cat Macula

In systemically injected cats, swollen mitochondria were found in Type I hair cell cytoplasm. The mitochondrial cristae disappeared in some of these, and transformation into a degenerative vesicular formation could be seen (Fig. 4) The nuclei of the Type I hair cells demonstrated chromatin aggregation. Other than these findings, all of the other structures of the Type I hair cells were normal Edematous and irregular appearance decrease in neurofibrils, and transformed mitochondria, as described above were found in the nerve chalice (Fig 5) The nerve chalice itself appeared swollen and edematous. Most of the Type II hair cells appeared intact, except for the existence of lysosome like electron-dense granules in the apical part of the hair cell All nerve endings of the Type II hair cells were morphologically intact.

The supporting cell structures, basement membrane, and adjacent nerves were all morphologically intact

In the topically injected animals, the macular destruction was much more severe than that in the macula from the systemically injected animals. The vestibular sensory epithelia in the topically injected animals differed from the systemically injected animals, in that all exhibited the same marked degree of degeneration. More cytoplasmic protrusions into the endolymphatic space were f und in the maculae however (Fig 6)

3 Squirrel Monkey Crista

The formation of vesicles and vacuoles in the cytoplasm was the significant finding. The mitochondria were swollen and reduced in number The Type I hair cell exhibited a shrunken appearance The cuticular plate free surface of the cell, and the sensory hairs were not significantly changed. The nuclei were shrunk with an aggregation of chromatin. The nerve chalices were severely swollen (Fig. 7) The numbers of neurofibrils were significantly decreased, and the mitochondria were swollen. Except for the synaptic junctions, th cytoplasmic membranes of the hair cell and the chalice were separated



Fig 4 A eprese tatiy ew of cat macula fter systemic dmi istration f lean cin anlf t Th swell ng f the nerve halice f the Type I hair cell (HC1) g ificant. The nu leu f the cell xhibits light hromatin ggreg tion. The Type II ha cell (HC2) i f l rs l ed VE indicates nerv endi g. 6800.



Fig. 5. The higher magnification view of Fig. 4 demonstrates the decrease of neurofibrils with slightly swollen and edematous appearance of the nerve chalice (*CL*). The mitochondria are swollen with ruptured cristae, sometimes vacuolated appearance (*arrows*) × 18,900.

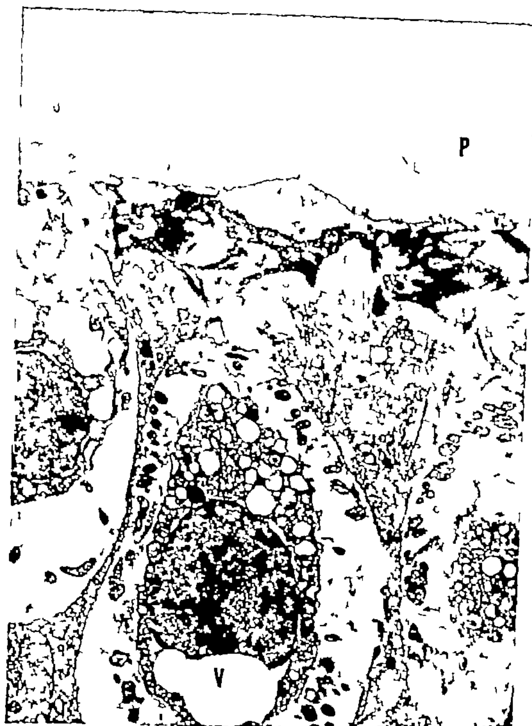


Fig 6 A view from the cat macul fter local dml istration f l mycin salf te (113 mg/ml) The sensory cell cyt pl am i filled with vacu les, nd the uclea membranes f th Type I ha cell (HC1) re separated, forming l rge vesicl (V) Degeneration i similarl severe the crist nd i def itel, more sev re when compared with th macula efter system injecti n. 6300.



Fig Electron micrograph demonstrates representative view f the squirrel monkey crista fter systemical i jection f omycin sulf t The destruction f the Type I hair cell is significant. Vesicles (*V*) nd vacuolizations in the cytoplasm as well as destruc tion of the mitochondria re seen The nucleu itself i transformed nd show chroma-ti aggregation. The nerv chalice *CL* xhibit sw ll ng nd the reductio of neuro-fibrils. A few swollen m tochondria with disorderly arranged cristae can lso be observed i the part of the nerv chal ce close t the se sory cell neck. 6300



Fig 8 A representati view from th squirrel monkey macul bt ined from the same animal s i Fig. 7 Not that except for the slightly d formed cytopl mic membrane, no significant pathological changes are found. HCI indicates hair cell Type I 5200.

The Type II hair cells were less involved morphologically but exhibited the same type of degenerative changes.

All the accessory structures such as supporting cells, basement membrane, and nerve myelin sheaths were morphologically intact

4 Squirrel Monkey Macula

The macula utriculi from the identical individual was far less involved when compared to the semicircular canal crista. The only finding in the macula was the slightly deformed cytoplasmic border of the hair cell Type I however no significant change in the nerve chalice could be observed (Fig. 8)

DISCUSSION

The differences in degree of damage in the crista and the macula (macula utriculi) both in the squirrel monkey and the cat, after systemic administration of viomycin sulfate was the important finding of the present investigation. This difference was clearly demonstrated at the electron microscopic level, and probably can be accounted for by different morphological vulnerability of the crista and macula, or the distance between these sensory end organs, and the secretory and absorbing epithelia. The latter theory is supported if endolymph does not intermix its components rapidly These findings are generally agreeable with those described after the administration of streptomycin sulfate in the squirrel monkey (Igarashi *et al* 1968) Nagaba (1968) noted an almost similar degree of morphological alteration in squirrel monkey canal cristae and utricular macula after systemic administration of streptomycin sulfate

On the other hand, the severity of the pathological changes was found to be not too much different after the local iomycin administration in the present study (Figs. 3 and 7) This is probably due to the fact that the antibiotic could reach the utricular macula easily after the local use

Th degree of destruction of hair cell in the canal cristae was very similar in both squirrel monkey and cat after systemic injection (Figs. 1 2 and) thus, this indicates that the primary pathology of the hair cells in the crista as observed is sufficient to create an ata ic gait in both of these species. Type I hair cells were more severely destroyed than Type II hair cells in all the canal cri tae and maculae investigated in the present study This is most probably due to a different cytometabolic process in these two types of hair cell, as was suggested by Wersäll & Hawkins (1962)

The severity f the vestibular end organ p thology was not h mogenous in the cri ta (F 1 and 2) no in the macula. In other words, some region of these nd o ns were more sev rely destroyed than other areas. This fact was previously reported by Lindeman (1969 *a* 1969 *b*) in his excellent surface specimen stud es On the oth hand, the difference between these two types of hair cell may exi t only at the initial stage of antibiotic intoxica

tion, as all hair cells will be destroyed after the increased administration of the drug

The report from Takenaka *et al* (1967) is the only other electron microscopic investigation reporting vestibular pathology after the systemic use of viomycin sulfate in experimental animals. Because their electron micrographs from guinea pig experiments demonstrate less destruction than our findings in the squirrel monkey and the cat (yet the total dose was comparable) it is suggested that the guinea pig's inner ear apparatus looks more resistant to viomycin sulfate. Resistance to antibiotic toxicity was previously mentioned in the rat which is another rodent, by Hawkins *et al* (1936) and by Riccio *et al* (1967) On the other hand, other investigators have found the guinea pig to be sensitive to ototoxic drugs such as kanamycin (Lundquist & Wersäll, 1966) and gentamicin (Lundquist & Wersäll 1967 1969)

The changes in the mitochondria and in the hair cell cytoplasm in Type I hair cells in the cristae were very striking after systemic viomycin injections Thus, the mitochondria may be the initial site of the destruction, and those were later transformed into vesicular formations with the disappearance of the cristae The pathological changes in the mitochondria are common findings among the different antibiotic ototoxicity as those were found after administration of neomycin sulfate by Takenaka *et al* (1967) after the systemic use of streptomycin sulfate by Duvall & Wersäll (1964) and by Hawkins (1967) in guinea pigs, in the cat by Wersäll & Hawkins (1962) and in the squirrel monkey by Nagaba (1968)

The free surfaces of both hair cells and supporting cells were generally intact after systemic injection however some cytoplasmic protrusion into the endolymphatic space was noticed These cytoplasmic protrusions were more prominent after the local injections (with isotonic 113 mg/ml solution) and were associated with more severe pathology The loss of sensory hairs which was described by Wersäll & Hawkins (1962) in the cat after streptomycin administration was not observed after systemic injection of viomycin in the present study but was seen to some extent after topical injection. These findings indicate that a high concentration of antibiotic when suddenly approached to the cell surface is destructive to the free surface (Lundquist, 1969)

The nuclear chromatin aggregation, which was previously described by Spoendlin (1967) in the acute streptomycin intoxication (local injection) in the cat was also quite apparent after topical injection in the present series however that could not be considered to be the specific finding after topical injection, as it was also found in the systemically injected squirrel monkey crista

The edematous and irregular appearances of the nerve chalices, and the reduction in the neurofibrils after viomycin toxicity is a unique finding of the present investigation. In all of the streptomycin ototoxicity reports by Duvall & Wersäll (1964) Hawkins (1967) and Spoendlin (1967) the nerve

endings were described to be intact. Only Farkashidy *et al* (1963) have described degenerative changes in nerve endings with swollen and clumping mitochondria due to kanamycin ototoxicity in the cat. Changes in the nerve chalice such as swelling, irregular cytoplasm and deformed mitochondria could be secondary to the advanced hair cell degeneration however the irregular appearance of the boundary between hair cells and nerve chalices in the macula, where the hair cells were least involved after systemic injection of viomycin, was observed both in the cat and in the squirrel monkey Therefore this finding may be a primary pathological change after viomycin sulfate injection Except for the synaptic junction, where the damage is severe, the cytoplasmic membrane of the hair cell and the chalice were separated. This is probably from the collapse of both sides. This finding is another interesting finding of the toxic effects of viomycin sulfate.

Cytoplasm of the dendrites leading to the nerve chalices was less compact after viomycin administration than in the normal condition This suggests that viomycin sulfate ototoxicity involves the peripheral neurons as well as the sensory hair cells. These dendritic changes, however could be secondary to the hair cell involvements.

Based on the above described morphological alterations in sensory cells and surrounding structures, it is reasonable to consider that the viomycin sulfate has reached the vestibular sensory end organs from the cell surface Despite the existence of pathology in the sensory hair cells, in the dendrites within the crista, and in some supporting cells, all the remaining structures which are located deeply i.e. close to the basement membrane, w re mor phologically intact

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ZUSAMMENFASSUNG

E wurde ine elektro enmikroskopische Untersuchung der Path logie des vestibularen Endorgan am Saimiri sciureus (squirrel mo k y) d der Katze vorgen mmen nach systemischer und lok ler Verabreichung n Viomycinsulf t. Wen Vi m insulfat yst misch i gespritzt wurde, zeigt sich bei den beiden Tierarten die ampullare krist mehr angegriffen h di utrikulare Makula. Andererseits zeigt n ich geri ge Unterschiede n der Pathologi we n die Droge lokal gewandt wurde V k l sation, esikulare Bild g, degenerierte Mitochondrien, nukleare Chr m t häufung usw wurden häufiger beobacl tet in Haarzellen om Typus I die stet stärker beschäd gt w ren I II arzellen m Typus II. Bei ystemischer Injektion war d fre Zelloberfläche gewöhnlich nicht betroffen. Der Nervkelch war geschw llen u d der Cyt plasmainhalt reduziert Dies lässt darauf schliessen, dass ach V omycinsulf t-I j ktion uch primäre Ei wirkung dieses Gebietes entsteht, d e irreguläre cytoplasmatische Grenze da gefunden wurde, wo die H rz ll m wen gsten betroffen waren

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ON THE CHANGES OF NYSTAGMUS CAUSED BY ULTRASOUND-PRODUCED FOCAL LESIONS IN THE MLF OF THE RABBIT

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The autho has studied on the changes of nystagmus caused by ultrasound-produced focal lesion in the MLF of the rabbit. Results obtained are summarized as follows

(1) A diminution of add cting novements on optokinetic and vestibular nystagmus was observed by destroyi g the MLF at the level of the rostral porti n f IV ventricle while a diminution of abducting movements on vestibular nystagmus, on the contrary was observed by destroying the MLF below the level of the trochlea nuclei

(2) In one rabbit, in which MLF was transected just below the level of the trochlear nuclei th so-called convergence nystagmus was transitorily observed.

(3) In the author's opinion, th difference between author's result and that of Bender & Weinstei (1944) seems t be due t the difference in species f the animals used.

Since Lorente de No (1933) reported that complete transection of the bilateral MLF (Median Longitudinal Fasciculus) in rabbits had produced no apparent disturbance in function of the ocul motor nucleus or in vestibular function, many experimental studies on the influence of lesions in the MLF or its neighbouring nerve fibers upon ocular movement or nystagmus have been reported by many workers up to the present day (Bender & Weinstein 1944 Bender & Shanzer 1964 Carpenter 1964 Sasaki, 1965)

On the other hand, it is clinically observed that lesions of the MLF rostral to the abducen nuclei produce specific disturbances of conjugate horizontal eye-movements without disturbances of ocular convergence.

This syndrome was named "Ophthalmoplegia internuclearis" by Lhermitte in 1922 Lutz in 1923 divided it into two types anterior and posterior internuclear palsies.

Since then, a number of clinical case-reports with autopsy findings and experimental data in animal concerning this syndrome have been accumulated. In spite of these reports, h wever problems still remain unsolved with respect to the classification into two types, and the exact localization of the lesions responsible for th occurr ce of this syndrome.

In order to provide s m inf rmation upon this problem the author produced in rabbits discret f cal lesions in the MLF near both the abdu

OKN stimulus 0.33 /sec.²–17/sec.–60 /sec.

r eye

l eye

CR

r eye

l eye

Fig. 1 Optokinetic nystagmus in No. 57 on 11th day after irradiation. Weakness of bilateral ocular adduction was clearly observed.



Fig. Histological changes in No. 57 on 33rd day after irradiation (Klüver-Barrera staining) *S.g.c.* substantia grisea centralis; *F.l.m.* fasciculus longitudinalis medialis; *M.Tr.* radix and nucleus mesencephalicus nervi V; *F.R.* formatio reticularis.

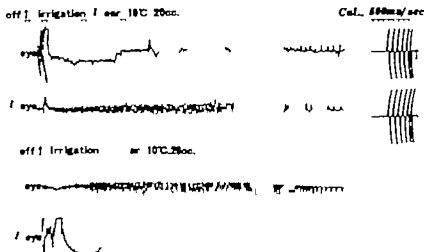


Fig 3 Caloric ystagmus in N 71 2nd day fter irradiation. Weakness f bilateral ocula abd ction was learly bserved, compared with Fig. 1

The lesion, 3 mm in length and 1 mm in diameter was of a spindle shape and well demarcated near the rostral portion of IV ventricle destroying total MLF of the right side.

Group B

In this Group as in Group A, postural abnormality and spontaneous nystagmus were not exhib ted. However positional nystagmus was observed in all cases. It is worthy of special mention that in No 67 dissociated nystagmus was observed. The typical case in this group was No 71 Here the weakness of bilateral ocular adduction in optokinetic nystagmus which appeared in Group A was not observed. On the contrary in caloric and rotatory nystagmus, weakness of bilateral ocular abduction was clearly observed on 3–5th day after irradiation (Figs. 3 and 4)

Immediately after irradiation, the left eye remained in neutral position, but the right eye deviated in adducting position, and, afterwards, the left eye also deviated slightly in adducting position.

In No 67 showing convergence nystagmus, positional nystagmus showed a type of dissociated nystagmus on 9–11th day This dissociation was more clearly observed in right-lateral position (Fig 5)

Hist logical changes in rabbit No 71 were as follows (Fig 6)

The lesion, 2 mm in length and 1 mm in diameter was located in the rostral porti n of the trochlear nucleus, in ol ing subtotal MLF of the left side total nucleus Gudden and partial bracchium conjunctivum.

Histological changes in N 67 showing transitorily dissociated nystagmus were as follows (Fig 7)

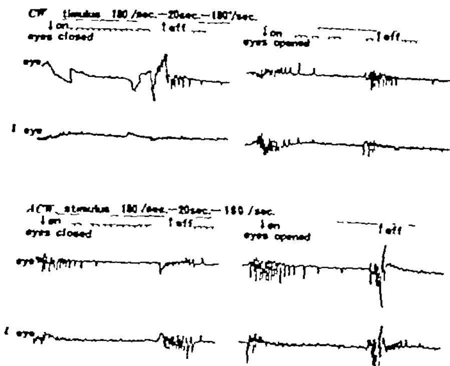


Fig 4 Rotatory nystagm In No. 71 3rd day fter irradiation.

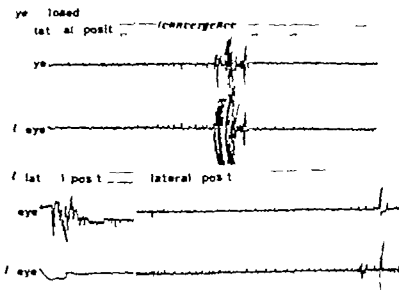


Fig 5. Positional nystagmus observed i N 67 on 9th day fter rradiation. Th upper figure shows ENG recording f co vergence nystagm nd the low show th t f dissociated nystagm s.

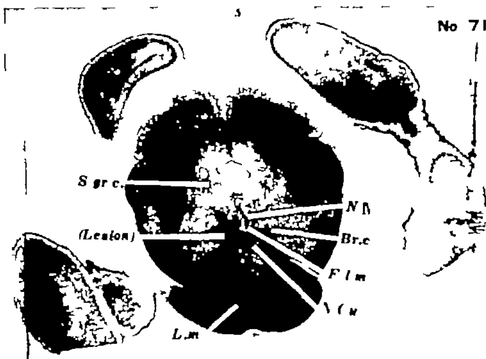


Fig 6 Histological changes in No. 71 on 12th day fter irradiati (Klüver-Barrera staining) *S.g .c.*, substantia grisea ce trali *L.m* lem iscu medialis; *VIV* ucle trochleari ; *Br.c.*, bracchi m co junctivum *F.l.m* fascicul longitudinali mediali ; *V.Gu.*, cleus Gudden.

The lesion, 2 mm in length and 1 mm in diameter was located at the level of the right trochlear nucleus, involving total MLF of the right side subtotal nucleus trochlearis and partial decussatio bracchii conjunctivi cerebelli

Summarized results of our experiment are shown in Table 1

COMMENT

As for the specific disturbances of eye movement caused by lesions of the MLF many reports have been accumulated up to the present day It is generally accepted clinically and experimentally that the lesions of the MLF produce disturbances of conjugate horizontal eye movements.

The author stereotaxically made localized destruction of the MLF by the use of focused high-frequency ultrasonic irradiation

High frequency ultrasound can produce sharply demarcated focal lesion in a desired part of the brain without any damage to the intervening nervous tissue along the pathway of the focused beam. The most significant result in author's experiments was that the so-called internuclear ophthalmoplegia and convergence nystagmus were observed

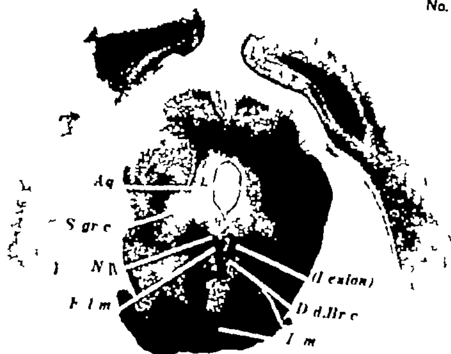


Fig. 7. Histological change in No. 67 on 29th day after irradiation (Klüver-Barrera staining). *Aq* aqueductus; *S.gr.c.* substantia grisea centralis; *N.IV* nucleus trochlearis; *F.l.m.* fasciculus longitudinalis medialis; *D.d.B.c.* decussatio brachii conjunctivi cerebelli; *L.m.* lemniscus medialis.

In Group A in which the MLF was transected at the level of rostral portion of IV ventricle, a diminution of adducting movements on optokinetic and vestibular nystagmus was observed. On the contrary, in Group B, in which the MLF was transected just below the level of the trochlear nucleus, a diminution of abducting movements on vestibular nystagmus was observed.

This variation in nystagmic reaction according to the difference in the transected level seemed to justify, to some extent, the classification into anterior and posterior types of internuclear ophthalmoplegia.

Bender & Shanzer from their experimental results obtained by destroying the MLF in the monkey stated that lesion of the MLF resulted in paralysis of adduction of ipsilateral eye and produced nystagmus in the contralateral abductor during horizontal deviation. In bilateral lesions of the fasciculus, the ocular dysfunctions were bilateral but in addition there was vertical *(usually upward)* nystagmus and *bilateral partial lid droop.*

The syndrome produced in the monkey corresponded closely to clinically observed cases in man. However, the situation in man has been obscure because of lack of suitable clinicopathologic material. In the few anatomically studied cases the clinical picture was beclouded by multiple signs, and the anatomical correlation by multiple or extensive lesions involving con-

siderable portions of the tegmentum of the brain stem besides the MLF For all that, most previous reporters agreed in considering that this syndrome was caused by a lesion in the MLF

But Madonick (1951) reported that in a case of bilateral ophthalmoplegia internuclearis anterior in a patient with a large tuberculome of the cerebellum, he could not find any lesion in the MLF or elsewhere in the brain stem.

As for the reason for the occurrence of the MLF syndrome in this case, he explained that it was reasonable to postulate that compression of the brain stem by a tumor in the posterior fossa might have caused functional interference in the MLF without anatomic changes.

Alexander & DeMyer (1966) has reported a typical case showing unilateral MLF syndrome with paralysis of adduction of the right eye on attempted gaze to the left and monocular horizontal nystagmus of the abducting left eye. He said that the findings agreed with the result of recent work in the macaque.

According to Walsh (1947) in anterior internuclear ophthalmoplegia the external rectus muscle functions normally in abduction but the internus of the opposite eye fails to contract in lateral eye-movements. The lesion is in the ascending fibers of the MLF In posterior nuclear ophthalmoplegia the internal rectus muscle acts normally in lateral movement during which the externus of the opposite eye fails to function. The lesion in this instance is in the descending fibers of the MLF From this consideration, he concluded that a lesion at the level of the trochlear nucleus results in anterior inter nuclear paralysis, and a lesion at the level of abducens nucleus results in posterior internuclear ophthalmoplegia.

Duke-Elder (1949) cites Lutz in the following definitions "In the an terior type, the externus on the side of the lesion functions normally (restrictedly) but the contralateral internus is paralysed for conjugate movements towards the side of the lesion and at the same time functions normally in convergence In the posterior internuclear type, on the other hand, both internal recti function normally in convergence and in lateral conjugate movements, but the externus on the side of the lesion is paralysed for conjugate movements"

Smith & Cogan (1959) proposed a different classification In the anterior type there is impairment of convergence associated with paralysis of the medial rectus on horizontal conjugate gaze toward the side opposite to the lesion. In the posterior type convergence is unaffected despite paralysis of the medial rectu in conjugate horizontal gaze It should be stressed that in their view there is weakness of the ipsilateral medial rectus muscle on hori zontal gaze in both types of internuclear palsy and there is characteristically a predominantly m noeular nystagmus seen in the abducting eye

Carpenter from hi animal experimentations, stated that the so-called anterior internuclear ophthalmoplegi may result only from lesions in the MLF in the vicinity of the abdu ens nucle and is probably due to interrup-

tion of secondary vestibular fibers projecting to both the abducens nuclei and to specific parts of the oculomotor nucleus

Moreover he said that the so-called anterior and posterior internuclear ophthalmoplegia probably represent variations of essentially the same syndrome In the anterior type paresis of ocular adduction is the most prominent finding, while weakness of ocular abduction is slight and manifested only by monocular nystagmus in the abducting eye In the so-called posterior *type the weakness of ocular abduction* occurs alone or presumably is greater than the weakness of ocular adduction It is difficult to understand how a posterior type could result from a lesion of the MLF without also producing an anterior type

The author s result was opposed to Walsh, i.e the levels of lesions occurring in the anterior and posterior types showed an adversative relationship, contrary to his theory

Moreover author s observation *that section of the MLF in rabbits* produced no paralysis of adduction but the eyes deviated in adducting position appeared to contradict the theory advanced by Bender & Weinstein that lesions of the MLF produce paralysis of ocular adduction.

According to Lorente de Nó, section of the MLF in rabbits produces no apparent disturbance in function of the oculomotor muscles or in vestibular function.

Bender & Weinstein state that this *difference* in the *experimental* results is due to the fact that the rabbit s eyes are laterally placed and have no need for horizontal conjugate movement as have the anteriorly placed yes of the monkey or man

However author s study revealed, contrary to Lorente de Nó s experiment that the section of MLF in rabbits produced disturbances in function of oculomotor muscles as mentioned above

In the author's opinion, the difference between author s result and that of Bender seems to be due to the difference in species of the animals used.

It is worthy of special mention that in No 67 the so-called convergence nystagmus was transitorily observed in positional nystagmus Since De-Monchy (1923) reported this specific syndrome many reports have been accumulated up to the present day However there are only a few cases with autopsy findings.

Generally it is said that the lesions responsible for the occurrence of this syndrome is due to the lesions situated in *the general area of the u per mid* brain This area comprises the posterior wall of III ventric p
with the corpora quadrigemina, and the tegmental part of t)
duncles including the periaqueductal gray Jung & K t
corded ENG curve of this nystagmus in a case with m
They stated that this syndrome consisted of paraly
ward, diminution of vertical optokinetic nystagmus,
gence nystagmus facilitated by intended upward gaz
of this syndrome, there are many hypotheses such as

(1924) Cogan (1959) and Segarra & Ojeman (1962) But, in the author s opinion, this syndrome may be due to the disturbance of hypothetical eye-centering system advocated by Bender (1955)

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I wish to express my gratitude to Professor H Ino, instructor Dr Y Kolke and Dr K. Mizukoshi fo thei advice at the Department f Ot laryngology Niig ta University School of Medicine

ZUSAMMENFASSUNG

Der Auto hat den F L m. (Fasciculus longitudinalis medialis) de Kaninchen mit dem hoch intensiven Ultraschall bestrahlt und die Veränderungen der Ny stagmusreaktion beobachtet, die durch die fokalen Läsionen hervorgerufen worden sind.

1) Die Verminderung der Adduktion Bewegungen in optokinetischen und vestibulären Nystagmus war durch die Zerstörung der F L m. an der Höhe des rostralen Teils des IV Ventrikel beobachtet worden während die Verminderu g der Abduktions-Bewegungen im vestibulären Nystagmus durch die Zerstörung der F L m. unter die Höhe des Nucleus trochlearis beobachtet war

2) Bei einem Kaninchen das die F L m. gerade u ter der Höhe des Nucleus trochlearis durchschnitten worden ist, ist vorläufig der sogenannte Konvergenz Nystagmus beobachtet worden.

3) Di Verschiede heit der Resultate zwischen dem Autor und Bender kommt nach des Autors Meinung von der Verschiedenheit der Art des gebrauchten Tieres.

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EUSTACHIAN TUBE FUNCTION ASSESSED WITH TYMPANOMETRY

A New Testing Procedure in Ears with Intact Tympanic Membrane

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A testing procedure allowing rapid and physiological determination f the tubal function in ears with intact ear drum is introduced. Testing results in normal as well as pathological ears are presented and discussed. The procedure seems to enable definition of tubal function disorders we have not hitherto been able to determine in clinical routine.

In ears with perforated tympanic membrane we can estimate the ventilating function of the Eustachian tube by using the aspiration method (Flisberg *et al.*, 1963) This can be done in clinical routine and gives precise and reliable information of the tubal function as documented by many authors (Miller 1965 Flisberg, 1966 Siedentop *et al.*, 1968 Holmquist, 1968) When, however the ear drum is intact we are in clinical routine today restricted to the use of such classic methods as the manoevres of Politzer Valsalva, Toynbee, tubal catheterising or by controlled overpressure technique which only give a rough and unphysiological estimation of the ventilating function of the tube (Ingelstedt & Örtegren, 1963) Using pressure chambers Thomsen *(1958) Ingelstedt & Örtegren (1963) and Ingelstedt et al.*, (1967) have designed methods by which it is possible to get quantitative and precise determination of the ventilating tubal function in cases with intact ear drums. However these methods are not adapted for clinical routine work. *The aim of this paper is to describe a new testing procedure* designed for clinical routine Negative middle ear pressure levels are established through the nose and Eustachian tube. By means of impedance measurement technique the way and extent of equalization upon swallowing are then recorded.

Equipment

Negative air pressur in the nose nd epipharynx is created with a pressure device (Fig. 1 milar in con truction to that of Ingelstedt & Ortegren (1963) The infl f changing the air pressure in the ear canal on the mobility and m t nd ti n f the tympanic membrane and the middle ear is investigated w th e thod named tympanometry During this the tympanic membra ded w th a hang of the air pressure in the

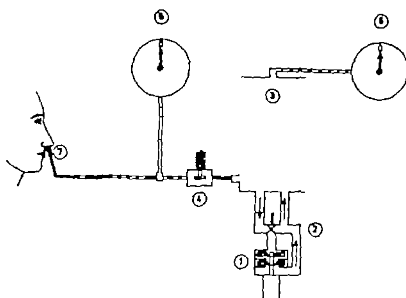


Fig 1 Air pressure device to create negative pressure in the nose and epipharynx. 1 [illegible]; 2 changing valve 3 air container 4 electromagnetic valve; 5 and 6, air pressure manometer 7 connection to the nose

canal. The sound level of the probe tone reflects the change of impedance of the tympanic membrane due to the air pressure load. The basic instrument for tympanometry is the impedance or intra aural reflex indicator A block diagram is shown in Fig 2. The two-pronged probe unit connected to the impedance indicator contains a receiver and a microphone The probe tone has a fixed frequency of 625 Hz. The microphone monitors the SPL in the ear canal between the ear drum and the cuff and the probe tone is adjusted to read 70 dB SPL. The air pressure change is obtained from a pump operated by an electric motor The variation of the air pressure in the ear canal is monitored by a pressure transducer connected to a manometer (EMT 33 and EMT 31 Elema-Schönander AB, Stockholm, Sweden) The recording of the reflected probe tone starts at a negative pressure of 200 mm H_2O The pressure then gradually rises to zero and up to +200 mm H_2O in altogether 45 sec. Manually it is possible to increase this range The tympanogram and the air pressure curve are continuously recorded on a Mingograf recorder For further details about the equipment see Lidén *et al* (in press) When the air pressure on both sides of the ear drum is the same the sound level recorded by the microphone reaches a minimum In this way it is possible to measure the air pressure in the middle ear by recording the ear canal pressure corresponding to the impedance minimum (Terkildsen & Thomsen, 19[illegible]9)

Performance

The testing procedure is divided into five steps, which are illustrated in Fig 3

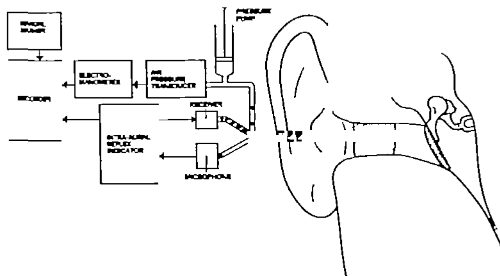


Fig 2 Block diagram showing the intra-aural reflex indicator and the air pressure pump.

A. By means of the intra-aural reflex indicator a tympanogram is recorded and the initial middle ear pressure evaluated.

B A negative pressure is created by the pressure device, which is connected to the nose. The patient is told to swallow When the soft palate closes a negative pressure of about −200 mm H_2O is established When, during swallowing, the tube opens, this negative pressure propagates to the middle ear which gives a rise in the sound level of the ear canal.

C. To evaluate the negative middle ear pressure reached a second tympanogram is made.

D The patient is told to swallow repeatedly When the tube opens the

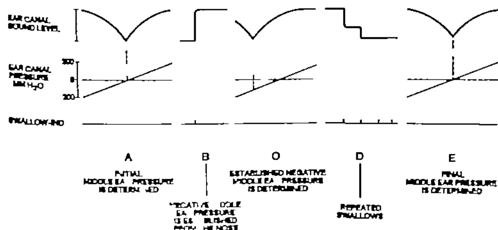


Fig 3. Schematic illustration f the different steps in the testing procedure.

middle ear pressure is stepwise equalized giving stepwise decrease of the ear canal sound level When swallowing does not give any further sound level changes the test is finished

E. In a third tympanogram the final middle ear pressure is registered

Comments

The difficulties in performance are few However in establishing the negative middle ear pressure sometimes repeated attempts must be made In certain cases it has also been observed that the established pressure has not been kept in the middle ear Even without swallowing a leaking through the tube occurs. In fact this phenomenon indicates good tubal function. Further in cases with nasal obstruction the test cannot be performed, otherwise a catheter in the nose is used to transmit the underpressure

MATERIAL

Normal cases

The testing procedure was used to determine the tubal function in 20 normal individuals. The ages were between 23 and 68 years in 11 men and 9 women. Only one ear was tested in each individual and the test was duplicated. In none of the cases was there any history of ear trouble All ear drum were normal as observed in the microscope and movable in Siegel's otoscope The hearing levels were within the range 0 to 20 dB (rel. to ISO standard 1964) for freq 500 1000 and 2000 Hz.

Pathological cases

Eleven cases were selected as pathological on the basis of a history of slight troubles with tubal function in rhinitis, allergic or infectious or in flying, diving, downhill driving or skiing In every other respect this group was identical compared with the controls

RESULTS

Normal cases

The results are illustrated in Fig 4 Each of the 20 ears was tested twice The middle ear pressure established through the nose was in the range of -100 to -250 mm H_2O These pressure levels could then be normalized with one or two swallows.

The reproducibility of the procedure is satisfactory In the repeated test it was possible to establish a negative middle ear pressure in the same range as in the first test. The difference between the two tests as for the pressure levels recorded after swallowing, was below 10 mm H_2O in 18 cases and 40 mm H_2O in 2 cases. The difference between the number of swall was in the two tests were one in a single case and none in the other 19 cases.

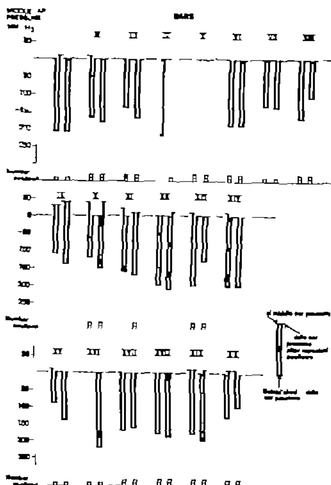


Fig 4 Indi idual testing results i normal cases.

Pathological cases

The results are illustrated in Fig 5 The air p essure established i middle ear was in the range of −90 to −200 mm H_2O In these ear testing results diverged compared with normal ears. First, if the midd pressure was equalized (cases I–IV) this demanded repeated swall (4–7) by these patients. Second, a residual pressure sometimes was re (cases V VI VII and IX) where further swallowings did not chan middle ear pressure In an ea lier report (Holmquist, in press) the has defined residual pressure as an indicato of decreased tubal f Third, in cases VIII X and XI the established middle ear pressure changed at all upon swallowing In case VIII it was possible by the test to d t rmine the residual pressure level In cases X and XI function of the tube was n t quantitated but the inability negative middle ear pressure wa judged as a sign of poor tubal

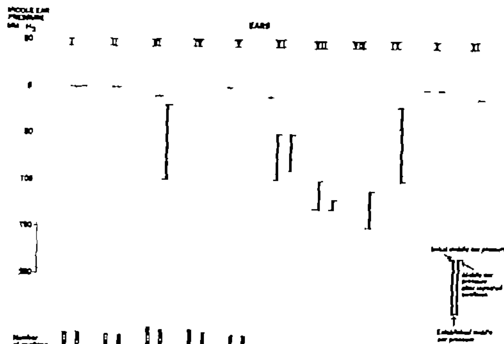


Fig 5 Individual testing result in pathol gical cases.

The tests were duplicated. In the second test it was possible to create a negative pressure of the same rang as in the first test (-90 to -200 mm H_2O) The difference between the two tests as for pressure level recorded after equalization were in four cases less than 10 mm H_2O in one case 10, in two 20 and in one 30 mm H_2O Thus even in this group the reproducibility is considered satisfactory

Comments

As is shown in Figs. 4 and 5 the middle ear pressure recorded by the tympanogram sometimes shows a positive pressure in the middle ear We know from investigations by Terkildsen & Thomsen, (1959) that the sound level minimum in the ear canal shows the middle ear pressure just at the moment of recording. However this is not the same as the middle ear pressure before the tympanogram is performed, as pointed out by Ingelstedt *et al* (1967) Changes of the middle ear pressure during the course of the tympanogram will occur e.g because of the movement of the ear drum and leaking of air through the tube These pressure changes are of less importance in this testing procedure but explain the positive pressure recorded, which is discussed later

DISCUSSION

Determination of the ventilating function of the Eustachian tube the ability to equalize negative middle ear pressure levels in ears with intact

tympanic membrane has not earlier been possible in clinical routine. The testing procedure introduced seems to fulfil such requirements, giving a more adequate and graded assessment of the tubal function than the classical methods commonly used. The method is simple to use and gives rapid and reliable information if and how the tube can equalize negative middle ear pressure in a similar way as does the aspiration method in cases with perforated ear drums. The procedure can be included in the routine in every clinic provided with equipment for tympanometry.

Duplicated tests and a thorough examination of the history of tubal troubles of the 31 cases here presented, show good reliability and validity of the procedure. Thus from the results it is possible to conclude that with the testing procedure introduced, it is possible to define certain slight tubal troubles, which we have not hitherto been able to determine in clinical routine.

As pointed out above, recordings with tympanograms sometimes show a positive middle ear pressure. This phenomenon occurs in normal as well as in pathological ears as shown in Figs. 4 and 5. A probable explanation is, that when a negative pressure is created in the ear canal, the movement of the ear drum increases the middle ear volume. Parallel with this an under pressure arises in the middle ear giving leakage through the tube. During the continuous change from negative to positive pressure in the ear canal, the ear drum moves again. When the original position is reached, the pressure in the middle ear as a consequence is on the positive side of zero, which is registered in the tympanogram. The problem of small changes of the middle ear pressure during tympanometry is interesting and complicated. Involved in the mechanism are several factors, such as the valve mechanism of the Eustachian tube, the volume of the middle ear and cell system and the mechanical properties of the ear drum. These problems deserve more research.

Technical advances in modern civilization introduce increased demands on tubal function, as in flying and diving. As a consequence of this many people complain of tubal dysfunction in stressed situations of this type. The method introduced provides the otologist with a possibility to determine such kinds of tubal dysfunction in clinical routine and enables him to give his patients good advice. It also seems obvious that people should be tested with the method described, before going into training for flying and diving.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben, die eine schnelle und physiologisch richtige Bestimmung der Tubenfunktion in Ohren mit intaktem Trommelfell erlaubt. Die Resultate der Funktionsbestimmungen bei normalen und patologischen Ohren werden präsentiert und besprochen. Die Art der Methode scheint zu ermöglichen, die Tubenfunktion in einem Grade zu bestimmen, der früher bei gewöhnlicher klinischer Routine nicht möglich war.

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THE DISPLACEMENT OF THE STAPES BY THE REFLEX OF THE HUMAN STAPEDIUS MUSCLE

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The integral characteristi of the human stapedius muscle is recorded The sound stimulus is applied to the contra-lateral ea Changes i th contractile state of the stapedius muscle as measured by the displacement f the head of the stapes acc rding to the sound-frequency and the intensity and the adaptati n of the muscle by successive stimuli for two min at 2000 cps and 120 dB SL are measured.

It is well known that an acoustic or non-acoustic stimulus induces the reflex contraction of the stapedius of both ears. Electromyography as a method of studying the properties of muscle is a differential method and lacks the ability to evaluate completely the properties of muscle adequately (Fisch & Schulthess, 1963 Djupesland, 1965) To date, however there has been no report concerning an integral and quantitative method of measuring the changes of the human stapedius muscle

We will report the integral measurement of the stapedius muscle by the displacement of the head of the stapes caused by contraction of the stapedius muscle This study was carried out under local anesthesia during surgery by touching the head of the stapes with the tip of a metal rod connected to a f ree-displacement pick up. In each case the opposite ear was normal An acoustic stimulation of the normal ear produced a contraction in the stapedius muscle of an experimental ear through the pathways used by the crossed stapedial reflex.

METHOD

Acoustic stimuli consisted of pure tones in the range from 1000 cps to 4000 cps using an audiometer (Rion, AA-33) One output of the audiometer was transmitted to an audiometer Booster (AE-26A) and amplified, and a headphone was pressed to the unoperated ear (Fig. 1) The maximum output of the audiometer Booster was 120 dB SL in the range of 1000–3000 cps and 115 dB SL at 4000 cps. Manual switching of the signals was used. One measuring terminal of a stainless steel rod 1 mm in width and 10 cm in length was bent like a hook and *another terminal* was *connected to a force* displacement pick-up (Nippon Kohden, SB-1T)

An FD pick up was connected to a pre-amplified (Nippon Kohden, RP-3)

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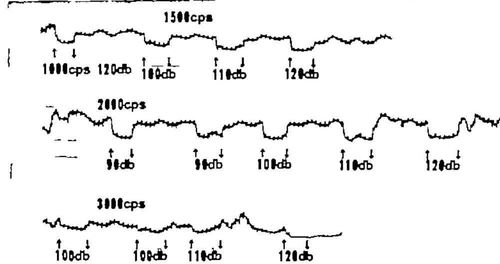
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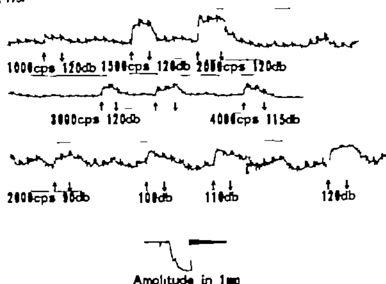


Fig. 2. The displacement f the head of the mobil stapes en loped by thi fibrou membrane resulting from 1000 cps t 4000 cps tones t several inten ity lev ls.

DISCUSSION

As reported by Fisch & Schulthess (1963) spontaneous discharges were observed under the noise of the operating room by contacting the measuring terminal to th head of st pes. It is said that these spontaneous discharges are equi alent to about 30 μV by electromyography and disappear completely 10 min after the intravenou injection of pent thal As in a report of Djupes-

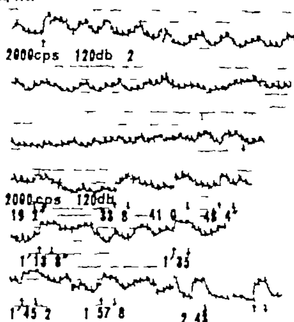


Fig 3 The daptation f the stapedi s resulti g from 2000 cps t ne t 120 dB for 2 min.

land (1965) the mobility of the stapes during surgery on the ear is produced by non-acoustic stimuli such as swallowing, clenching the teeth, opening the mouth, vocalization and eye closure but these stimuli lack in their ability to be quantitative indices of the mobility of the stapes.

The measurement of the displacement of the head of the stapes caused by contraction of the stapedius muscle was clearly characteristic for each frequency and the greatest response was observed at 2000 cps in the range from 1000 cps to 4000 cps (Fig. 2) As shown in Fig 3, the adaptation of the stapedius muscle showed spontaneous discharges only at an early period during successive stimulation for 2 min at 2000 cps and 120 dB SL, which is considered to be an interesting phenomenon.

ZUSAMMENFASSUNG

Die integralen Kennzeichen des menschlichen Musculus stapedius wurden registriert. Ein akustischer Stimulus wurde an einem Ohr gegenüber einem Testohr erzeugt. Veränderungen in der Kontraktilität des Musculus stapedius, gemessen an der Verschiebung des Stapeskopfes nach Frequenz und Tonstärke und die Anpassung des Muskels bei aufeinanderfolgenden Stimuli während einer Dauer on 2 Minuten bei 2000 Hz und 120 dB wurden gemessen

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X-RAY IRRADIATION OF THE INNER EAR OF THE GUINEA PIG

Early degenerative changes in the vestibular sensory epithelia

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X-ray irradiation in single doses was applied to the inner ear of guinea pigs. The vestibular sensory epithelia were examined by light microscopy using the surface specimen technique.

1 The degeneration of sensory cells was found after exposure to 6000 R and 7000 R

2 The sensory cells in the periphery of the cristae ampullares and the maculae sacculi and utriculi were most susceptible to damage

3 The number of degenerated sensory cells was slightly higher in the cristae ampullares and the macula utriculi than in the macula sacculi.

4 Most of the degenerated sensory cells seemed to be of the type II

5. The first sign of degeneration was a coarse granulation of the nucleus.

6 Further signs of degeneration were pyenosis of the nucleus and a spiderlike structure in the cuticular region.

7 The first sign of degeneration was found after an interval of three hours from the exposure

8. Completely degenerated cells were found after an interval of eighteen hours from the exposure.

9. No pathological changes were noted in the supporting cells.

10 Exposure to 2000 R and 4000 R was not followed by visible changes.

The effect of ionizing radiation on the vestibular part of the inner ear has been investigated both by functional and histological methods. The most common symptoms following the irradiation of animals are disturbances of the equilibrium (Ewald 1905 Chilow 1927 Gerstner *et al* 1954 Berg & Lindgren 1961 Levy & Quastler 1962) In rabbits, McDonald *et al* (1963) found reduced post rotatory nystagmus after local exposure of the inner ear to an alpha particle beam Spontaneous nystagmus has also been observed but rarely (Marx 1909)

Histological studies have revealed degenerative changes in the vestibular part of the labyrinth (Marx, 1909 Chilow 1927 Berg & Lindgren, 1961 Levy & Quastler 1962 Kelemen, 1963) However no detailed information is given by these authors with regard to possible difference in vulnerability between the sensory and the supporting cells, between the type I and type II

Table 1 *Number of guinea pigs doses of irradiation and intervals between irradiation and sacrifice*

	2000 R	4000 R	6000 R	7000 R
1 week	1	2	4	3
3 hours				5
6 hours				5
18 hours				3

sensory cells, between the different regions within the cristae and the maculae, and between the individual sensory areas (the maculae sacculi et utriculi, the cristae ampullares) Neither do we know when the degeneration starts. The present investigation was undertaken to shed more light on these questions.

MATERIAL AND METHODS

Twenty three guinea pigs were irradiated with single doses of 2000 R, 4000 R, 6000 R and 7000 R They were sacrificed 3 hours, 6 hours, 18 hours, and 1 week after the irradiation The number of animals in each group and the doses applied are given in Table 1 The source of radiation was a Siemens Stabilipan roentgen apparatus operated at 200 kV 12mA and equipped with a Thoreaus filter II (0 8 mm Sn, 0.25 mm Cu, and 1 mm Al.) Focus-skin distance was 232 mm Dose rate at the site of the inner ear was 200 R/min The X ray dose was delivered to the left half of the skull through a rectangular hole (20 mm × 1 mm) in a lead sheet (thickness 10.5 mm) which also shielded the rest of the animal For further details regarding the experimental animals, the irradiation conditions and the dosimetry the reader is referred to a previous paper (Winther 1969 *a*)

In the papers cited in the introduction the histological examination of the irradiated inner ears was confined to light microscopy of sections. In the present investigation the surface specimen technique (Engström *et al* 1966 Lindeman, 1969 *b*) was applied combined with conventional histological examination

The guinea pigs were decapitated and the lower jaw removed. The temporal bones were isolated from the skull and a wide opening was made in the vestibulum. The utriculus and the sacculus were opened, and within 2–3 min after the death of the animal the labyrinth was irrigated with the fixation fluid (1.5% veronal buffered osmiumtetroxide solution at a temperature of 4 C) through a thin plastic catheter connected to a 10 ml syringe. The fixation time was 1–2 hours. They were then washed for / hour in physiological saline. The further preparation took place under binocular dissecting microscope The crystal membrane of the sacculus was washed away The sensory epithelium wa removed *in toto* and mounted in glycerine After removal from the temporal bones the utriculus and the three ampullae



Fig. 1. Surface specimen of crista ampullares. The sensory epithelium is peeled off the underlying tissue and mounted in glycerine. Phase contrast micrograph. Magnification 85. Obj. 25 Oc. 8.

were treated in the same way (Fig. 1). When applying phase contrast microscopy on these specimens a birds eye view of usually nearly all of the sensory and supporting cells may be obtained and by focusing one can have "optical sections" of the sensory hairs, and of the nuclear and the cytoplasmic portions of the cells (Fig. 2).

In six animals the degenerated cells were counted within groups of a hundred sensory cells. Since the number of sensory cells per square unit is higher in the periphery of the maculae and the cristae ampullares than in the striolae and in the central areas of the cristae ampullares (Lindeman 1969 *a* and *b*) these regions were examined separately.

In two animals the maculae sacculi and utriculi and the cristae ampullares were embedded in Araldite sectioned and examined by phase contrast microscopy.

In all animals except one the left and the right temporal bones were prepared and examined in the same way, the right side serving as control.

RESULTS

Normal Anatomy—Controls

The sensory cells in the vestibular part of the labyrinth are located in the maculae sacculi and utriculi and in the cristae ampullares superior, lateralis

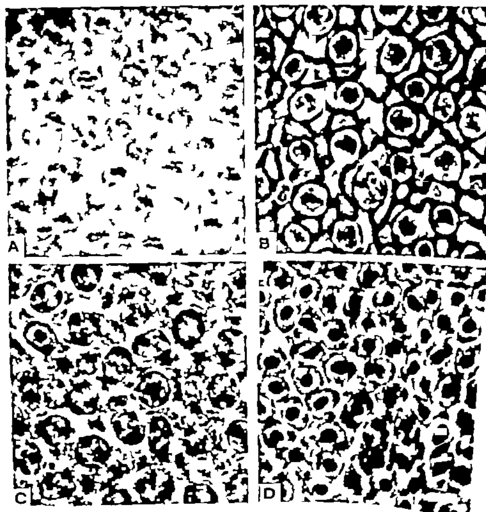


Fig 2 (*a–d*) Phase contrast micrographs from the macula utriculi at four different levels ( ) the hai t fts, (*b*) the cuticular region, (*c*) the plane sensory cells, and (*d*) the 1 1 f the supporti g cells. M gnification ... Oc. 8. ... 150

and posterior The sensory epithelium consists of sensory ce... ing cells. There are two types of sensory cell (Wersäll, ... Wersäll, 1958) The type I and the type II sensory cells diff... and nerve supply The type I cells are bottl shaped. The ... fibres begin with a calyx which covers most of the cell bo... II cells are cylindrical They are innervated by numerou... endings of various size For further details the reader ... man (1969 *a* and *b*)

In the surface specimen technique preparations a total ... five spiderlike structures can be observed among the ...

sensory[1] and supporting cells when the cuticular region is in focus. On the basis of the observation of different stages of degenerating sensory cells in normal and experimental animals Lindeman (1967 and 1969 *b*) interprets the spiderlike structures as remnants of degenerated sensory cells presumably formed by shrinkage of the cytoplasm. Accepting Lindeman's interpretation, the spiderlike structures are in the following described as *degenerated sensory cells*.

In none of the 22 normal controls did the total number of degenerated sensory cells in each sensory area exceed five.

General Post Irradiation Symptoms

A dose of 7000 R was given to 16 animals. Eight of these animals showed disturbances of the equilibrium ranging from slight instability when walking to inability to stand upright. Six out of these eight animals deviated or fell toward the left irradiated side, while two deviated toward the right. The symptoms were evident about an hour after completion of the irradiation. No appreciable improvement was observed even after a week. Spontaneous nystagmus was not observed. Positional nystagmus occurred in three of the animals which showed disturbance of equilibrium. The direction of the nystagmus alternated but no definite correlation between the position and the direction of the nystagmus could be detected. Vestibular symptoms were not observed in animals which received less than 7000 R.

In the animals sacrificed one week after the irradiation damage to the skin consisting of slight edema and epilation in the irradiated field was observed in the 6000 R and 7000 R groups. All other animals remained in good general condition until they were sacrificed. No gross pathological changes were noted in the middle or inner ears.

Sensory Cells one Week after Irradiation

2000 R (1 animal) and 4000 R (2 animals)

The number of degenerated cells were within the range observed in the controls.

6000 R (4 animals) and 7000 R (3 animals)

In all these animals except two of the 6000 R group, a marked increase was found in the number of degenerated cells (Table 2, Fig. 3). In one of the two exceptions the specimens were examined by the surface specimen technique while in the other the specimens were sectioned. In the former, however, the total number of degenerated cells was clearly higher than in the controls. In the latter, degenerated cells could not be found.

[1] Total number in macula sacculi 6900, maculi utriculi 8100, crista ampullae lat. 6000 (Lindeman, 1967).

Table 2 *Percentage of degenerated sensory cells in the periphery and in central areas of the cristae ampullares respectively the striolae in guinea pigs following irradiation with single doses of 6000 R and 7000 R (survival time one week)*

	Macula sacculi		Macula utriculi		Crista amp. sup		Crista amp. lat.		Crista amp post.	
Dose	Striola	Periphery	Striola	Periphery	Central area	Periphery	Central area	Periphery	Central area	Periphery
6000 R	0–1	9–19–1 5	0–1	11 1 –11–9	—	—	2	8	1	4
6000 R	2	6	1	5	2	16	4	13	1	10
6000 R	1	1	0	1	0	2	0	0	0	1
7000 R	0	2	—	—	5	19	—	—	5	13
7000 R	0	4	2	25	1	20	3	14	0	7
7000 R	0	3	3	16	4	15	3	16	5	"0

As a rule the number of degenerated cells was slightly higher in the cristae ampullares and the maculae utriculi than in the maculae sacculi. No marked difference was found between the maculae utriculi, the cristae ampullaris superior lateralis and posterior (Table 2)

A marked difference was evident between the peripheral portions of the sensory areas and the central regions of the cristae ampullares, respectively the striolae. The percentage of degenerated cells in the periphery was usually about four times that found in the central regions of the cristae ampullares and the striolae (Table 2)



Fig 3 Phase contrast micrograph from the periphery f the crista ampullaris posterior of an nimal irradiated with 6000 R X-rays in one singl dose nd sacrificed one week following the irradiation. Focal pl ne t the le l of the cuticula plates showing many spiderlike structures (arrows) Magnification 980, Obj 100, Oc. 8.

sensory[1] and supporting cells when the cuticular region is in focus. On the basis of the observation of different stages of degenerating sensory cells in normal and experimental animals Lindeman (1967 and 1969 *b*) interprets the spiderlike structures as remnants of degenerated sensory cells presumably formed by shrinkage of the cytoplasm. Accepting Lindeman's interpretation, the spiderlike structures are in the following described as degenerated sensory cells.

In none of the 22 normal controls did the total number of degenerated sensory cells in each sensory area exceed five.

General Post Irradiation Symptoms

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Dose	Striola	Periphery	Striola	Periphery	Central area	Periphery	Central area	Periphery	Central area	Periphery
6000 R	0–1	9–19–1–5	0–1	11 1 –11–9	–	—	2	8	1	4
6000 R	2	6	1	5	2	16	4	13	1	10
6000 R	1	1	0	1	0	2	0	0	0	1
7000 R	0	2	—	—	5	19	—	—	5	15
7000 R	0	4	2	23	1	20	2	14	0	7
7000 R	0	3	3	16	4	15	3	16	5	20

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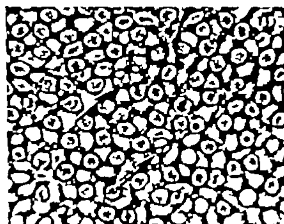


Fig 3 Phase contrast micrograph from the periphery of the crist amp llari posterior of animal irradiated with 6000 R X-rays i one si gl dose nd sacrificed one week following the irradiation. Focal plane t the lev l of the cuticula plates showi g ma spiderlike structures (arrows) Magnification 950, Obj 100, Oc. 8.

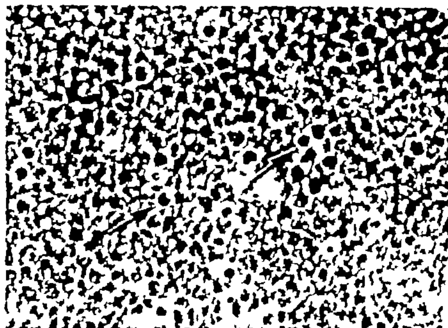


Fig. 4. Phase contrast micrograph from the macula sacculi of an animal irradiated with 7000 R X-rays in one single dose and sacrificed 3 hours following the irradiation. In many of the peripheral sensory cells the nucleus is pycnotic and surrounded by a highly refractive zone (arrows). Magnification 470, Obj. 40, Oc. 8.

Sensory Cells Three, Six and Eighteen Hours after Irradiation

7000 R (13 animals)

Three hours after the completion of the irradiation, cells with a coarsely granulated nucleus bordered by a highly refractive zone were observed in the periphery of the cristae ampullares and the maculae (Figs. 4 and 5). Some of these cells showed the spiderlike degeneration structure in the cuticular region, often with a tuft of sensory hairs although these hairs appeared distorted. In others, however, with the same nuclear changes the cuticular region appeared normal.

Six hours following the irradiation, slight to marked pycnosis of the nuclei of the sensory cells was noted in the periphery of the sensory areas. All of these cells had the spiderlike structure in the cuticular region and many of them were surrounded by a highly refractive zone. In addition to the cells with a pycnotic nucleus, a number of cells displayed the characteristic changes observed three hours after the irradiation. In surface specimen preparations it was not possible to determine the type of the sensory cell with nuclear changes. This question could neither be conclusively answered by microscopy of sections although there was some evidence that most of them were of type II.

Eighteen hours following the irradiation a picture similar to that observed one week after the irradiation was found, although at this time a small

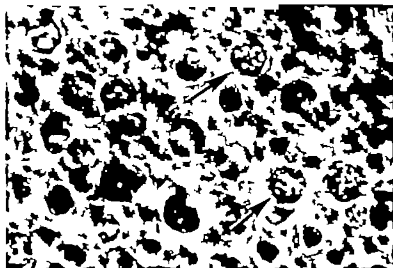


Fig 5. Phase contrast micrograph from the periphery of the macula sacculi f [illegible] imal irradiated with 7000 R X-rays i ne singl dose nd sacrificed 3 hours following the irradiation. Focal plane t the lev l of the sensory cell uclei. Many f the nuclei ha coarse granul tion (arrows) i co trast t ormal uc i with nearly homogenou appearance M gnification 1680, Obj. 100, Oc. 10.

number of sensory cells with a coarsely granulated or pyenotic nucleus was occasionally noted

Supporting Cells

No pathological changes were noted in the supporting cells following irra diation.

DISCUSSION

The selection of the experimental animals, the irradiation conditions and the dosimetry is discussed in a previous paper which is referred to (Winther 1969 a)

The surface specimen technique was found to be of great advantage in this study where a survey of a great many sensory cells and an exact localiza- tion of damaged or degenerated cells were intended. In this respect it is superior to the conventional method of histological sections which appeared to be insufficient for identification of degenerated cells in experimental animals with survival time of one week. In secti ns from animals sacrificed 6 hours after the irradiation, however cells with the characteristic nuclear changes were easily recognized. Thi fact made the investigation of sections a valuable supplement to the surface specimen technique The find [illegible] f a tuft of sensory hairs in cells with a typical spiderlike structure in the cu- ticular region and a granulated or pyenotic nucleus in the same cell defin

tely proved that the spiderlike structure represents a degenerated sensory cell

A slight difference was found in the vulnerability of the various sensory areas which was indicated by a slightly higher percentage of degenerated sensory cells in the cristae ampullares and the maculae utriculi than in the maculae sacculi. A similar pattern was found by Chilow (1927) following radium irradiation of the inner ear of the cat. Histological examination was performed several months after the irradiation and consequently is his investigation not quite comparable to the present one. A relative resistance of the macula sacculi to noxious agents is also indicated by the finding of the same pattern in streptomycin treated cats (Berg, 1951, Wersäll & Hawkins, 1962), squirrel monkeys (Igarashi *et al.* 1966) and guinea pigs (Lindeman 1967). At present no explanation for these findings can be given. They are, however, not in agreement with the assumption that the *macula sacculi as a phylogenetically younger part of the vestibular labyrinth* might be more susceptible to damage (Schuknecht *et al.* 1965).

A definite pattern of degeneration was found within the individual sensory areas. The percentage of degenerated sensory cells in the peripheral zones of the maculae and the cristae ampullares was approximately four times that of the striolae and the central areas of the *cristae ampullares* (Table 2). In this respect the effect of the X rays are unlike that of the antibiotics of the streptomyces group which mainly affect the sensory cells in the striolae and the central areas of the cristae ampullares (Berg, 1951, Igarashi *et al.* 1966, Lindeman, 1967). The type I cells are more susceptible to antibiotics of the streptomyces group than the type II cells (Wersäll & Hawkins, 1962, Spoendlin 1966, Lindeman 1967). An explanation for the pattern of degeneration caused by the antibiotics of the streptomyces group may be that the type I cells are more commonly located in the central area of the cristae (Wersäll 1956, Spoendlin, 1965) and in the striolae (*Engström & Wersäll 1958, Spoendlin 1965, Lindeman 1969 a and b*). An explanation of the pattern of degeneration found after X ray irradiation might be given along a similar line. Although the type of the normal sensory cell could usually be determined with great certainty in total mount and by light microscopy of sections, the type of the degenerating sensory cells could not always be determined by the same method owing to a slight swelling of the cell. There was some evidence, however, that the majority of the degenerating cells were of type II. Electron microscopic investigation in progress seem to confirm this observation (Winther 1969 *b*). The predominance of the degenerated sensory cells in the periphery might be explained by the more common location of the type II cells in the most peripheral zone of the maculae (Engström & Wersäll, 1958, Spoendlin 1966, Lindeman 1969 *a* and *b*) and of the cristae ampullares (Wersäll 1956, Spoendlin 1966, Lindeman, 1969 *a* and *b*). The pattern of degeneration might also be due to other morphological and biochemical differences between the more and the less affected area. It should be remembered in the

connection that the density of the cell population is highest in the periphery (Lindeman 1969 *a* and *b*) The sensory cells of the periphery are innervated by thin and medium thick myelinated fibres whereas the central area of the cristae ampullares and the striolae also are supplied by thick myelinated fibres (Cajal, 1908 Lorente de Nó 1926 Wersall, 1956 Lindeman, 1969 *a* and *b*) The intereplthelial nerve plexus which runs parallel to the surface in the lower half of the epithelium is richest at the periphery (Lorente de Nó, 1926 Poljak, 1927 Wersäll 1956 Lindeman, 1969 b) This nerve plexus is probably formed by efferent nerve fibres (Engström, 1958 Smith & Rasmussen, 1967) A higher content of acetylcholinesterase is demonstrated in the periphery (Hilding & Wersäll, 1962 Nomura *et al.*, 1965) It remains unknown whether and how these morphological and biochemical factors could affect the radiosensitivity of the sensory cells. At present it is neither possible to explain the difference in radiosensitivity between the sensory and the supporting cells nor between the type I and the type II sensory cells.

Impaired vestibular function was found in eight of the 16 animals irradiated with 7000 R. This might be due to pathological changes in the labyrinth, in the brain stem including the vestibular ganglia, and in the cerebellum all of which were in the field of the irradiation Definite correlations were not found between the symptoms and the number of degenerated sensory cells These observations support the assumption that the disturbance of the equilibrium was not a pure labyrinthine sign. In this respect it is interesting to note that the granule cells of the cerebellum are very sensitive to ionizing radiation (Schümmelfeder 1962 Zeman *et al* 1968)

No signs of hemorrhage or exudation were observed in the middle or internal ear contrary to the findings in many previous investigations (Chilow 1927 Thielemann, 1928 Ivanov 1957 Berg & Lindgren, 1961 Levy & Quastler 1962 Kelemen, 1963) This discrepancy might be explained partly by different irradiation conditions, partly by different methods of sacrificing the animals and partly by different intervals between the irradiation and the examination (Winther 1969 *a*)

The degeneration of the sensory cells may be caused by a direct action of X rays on the cells or indirectly by a primary action on the blood vessels thus interfering with the supply of oxygen and nutritients. A third possibility is a combination of these factors. Preliminary investigation of the blood vessels of the inner ear did not reveal any major pathological changes The functional state of the vessels, however cannot be judged from histological specimens alone (Kreyberg, 1929)

ZUSAMMENFASSUNG

Die Wirkung einer Einzeldose von Röntgenstrahlen auf den vestibulären Teil des inneren Ohres von Meerschweinchen wurde lichtmikroskopisch an Präparaten untersucht, die nach der Methode von Engström hergestellt waren.

1. Die degenerativen Veränderungen wurden nach Dosen von 6000 R und 7000 R beobachtet
2. Die grösste Anzahl von degenerativen Sinneszellen wurde in der Peripherie von der Cristae ampullares und der Maculae sacculi sowie utriculi beobachtet
3. Die Anzahl der degenerativen Sinneszellen war etwas grösser in der Cristae ampullares und der Maculae utriculi als in der Maculae sacculi.
4. Die meisten degenerativen Sinneszellen schienen von der Type II zu sein
5. Das erste Anzeichen der Degeneration war eine grobe Granulierung der Kerne
6. Weitere Anzeichen einer Degeneration waren Pycnosis von den Kernen und eine spinnenähnliche Struktur in der Cuticular Region.
7. Die ersten Kernveränderungen wurden 3 Stunden nach der Bestrahlung festgestellt.
8. Die ersten vollständig degenerierten Zellen ergaben sich 18 Stunden nach der Bestrahlung
9. Keine pathologischen Veränderungen wurden in den Stützzellen beobachtet.
10. Dosen von 2000 R und 4000 R verursachten keine sichtbaren Veränderungen.

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THE NORMAL ELECTROMYOGRAM IN HUMAN VOCAL MUSCLES

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By means of the transcutaneous approach previously used in retrograde inspection of the larynx a method for electromyographic examination of the vocal muscle in man has been developed which usually permits identification of some twenty motor unit potentials from each muscle without causing apparent discomfort to the subjects. Data obtained in recordings from eighteen normal muscles in eight healthy subjects and five patients with unilateral vocal cord pareses are presented.

During quiet breathing a sustained activity was present in several motor units. In some units the discharge frequency was independent of the respiratory phases, in other units the frequency varied with respiration, both expiratory and inspiratory units being found in this latter group.

The motor unit potentials displayed one up to seven phases, the majority being di- or triphasic. Five per cent were polyphasic. The mean potential duration was 3.70 ± 1.01 msec (S.D., $n = 409$). Two age groups, 20–30 respectively 40–60 years of age, displayed a small though significant difference in mean potential duration. The mean amplitude of the potentials was 0.41 mV ($n = 323$) and the 10th and 90th percentiles were 0.15 respectively 0. 0 mV. During deglutition the maximal amplitude of the interference pattern usually varied in the range 1.5–2.5 mV.

From a series of investigations of the electromyographic activity in the intrinsic laryngeal muscles of patients with vocal cord pareses it became apparent that available data on the normal activity were not sufficient to permit reliable diagnostic criteria to be established. This may be attributable to the difficulties encountered in the identification in single muscles of a sufficient number of individual motor unit potentials since most of them are concealed by a continually sustained electrical activity (cf. Weddell et al., 1944). Besides, the respiratory movements of the vocal cord may cause shifts in the position of the recording electrode relative to the muscle fibers. Thus, in the most extensive electromyographic study on the intrinsic laryngeal muscles published so far (Faaborg Andersen, 195) only few motor unit potentials could be identified in each muscle.

The experiments to be described were undertaken in an attempt to obtain more extensive information on the normal electromyographic activity of the vocal muscle. By means of a transcutaneous approach previously employed in retrograde inspection of the larynx (Mårtensson et al. 1964) a recording

procedure has been developed which usually permits identification of more than 20 motor unit potentials from each muscle In the present paper this procedure will be described and data given on the electromyographic activity in the normal vocal muscle As will be shown in a subsequent paper typical deviations from the normal pattern in vocal cord pareses can usually be disclosed by means of these data.

METHODS

Eight subjects with no known laryngeal lesions or diseases and five patients with unilateral vocal cord paresis were studied. The experiments were performed on the vocal muscle (the internal thyroarytenoid) bi laterally or on the healthy side respectively

For the recordings a concentric needle electrode (Disa, type 9013 K 32) was used, of a length of 43 mm, outer diameter 0.65 mm, leading-off surface of the platinum core 0 03 sq.mm and bevel angle 15 The recordings were made between the center core and the outer needle. A large surface electrode placed on the chest wall midway between heart and larynx connected the subject to earth, thus reducing the interference from the ECG.

The action potentials were fed to a differential amplifier with an input impedance of 3 megohms and essentially linear frequency response between 4 Hz and 10 kHz. The output was connected to an audio monitor and a loud-speaker as well as to an oscilloscope on which the potentials were displayed at sweep velocities of 2 or 5 mm per msec and photographed on moving film or paper (velocity 50 mm/sec) Analyses of the shapes, durations and amplitudes of the potentials were made only on those from which at least two identical recordings were obtained and whose onset and end could be reasonably well defined.

In order to reduce secretions in the respiratory tract 0.25 mg atropine sulphate was injected subcutaneously about 15 min prior to the experiment thus markedly diminishing the irritation and coughing. After anesthetizing the skin overlying the cricothyroid membrane by injection of 1–2 ml of 1% lidocaine solution with epinephrine the membrane was penetrated by a thin cannula through which 0.5–1 ml of 4% lidocaine solution was sprayed over the subglottic mucosa As soon as the brief initial irritation thus caused had subsided, the electrode was inserted through the skin and the midline of the cricothyroid membrane when this had been pierced, which can be felt without difficulty the needle was advanced another 1–2 mm The subject was then requested to hold his breath after an inspiration thus bringing the vocal cords into an adducted position. Before the needle was further ad vanced it was angled maximally upwards but only slightly laterally in order to increase the possibilities of selective penetration of the vocal muscle when the electrode was inserted and to minimize the risks of unintentional recording from the external thyroarytenoid muscle As soon as the electrode makes contact with a vocal cord, a change in the electrical resistance at the

tip is displayed on the oscilloscope screen From this position the needle was advanced approximately another 10 mm the electrode is calculated to penetrate the lower surface of the vocal cord at its midpoint and to proceed from there toward the muscular process of the arytenoid cartilage

The recordings started with the electrode at maximal depth, and then the electrode position was altered by rotation and gradual withdrawal of the needle Once this had started care was taken not to bring the needle back deeper into the muscle since this was apt to cause discomfort to the subject As a rule each vocal cord was impaled only once When the subject began to cough or show other signs of irritation usually after 15–20 minutes recording, probably because the surface anesthesia began to wear off the recordings were interrupted and the needle withdrawn

One of the subjects developed a slight hoarseness and irritation in the larynx for a couple of days following the experiment In another subject indirect laryngoscopy performed immediately after the recording revealed a slight subcutaneous bleeding (1 mm in diameter) on the upper surface of one of the vocal cords which had however disappeared the next day With the exception of these two cases no untoward effects of the experiments were observed

RESULTS

Activity pattern during quiet breathing

A characteristic feature of the EMG pattern from the vocal muscle is that the electrical activity is maintained in spite of any efforts to attain complete relaxation (cf Weddell *et al.*, 1944) This activity consisting of continuous discharges from several motor units with fibers more or less distant from the electrode within its pick up area, makes it difficult to identify individual units since the onset and end of the potentials may be concealed by baseline disturbances and since potentials from different units may interfere with one another In one and the same muscle this resting activity varied considerably at different electrode positions. In most areas tested, too much activity was picked up to permit identification of individual motor unit potentials but by minimal electrode movements some needle positions could usually be found at which one up to three different potentials were clearly discerned (Fig 1) Except in one case in which no motor unit potential could be unequivocally defined in any position tested it proved to be possible to identify at least 20 units from each muscle

The discharge frequency of the units usually varied from 10 to 20 per sec, but although efforts were made to avoid activity in the intrinsic laryngeal muscles, a comparatively large number of units discharged at 40/sec which is a remarkably high rate considering that the average maximum frequency during phonation has been reported to be of about the same order (Faaborg-Andersen 195[illegible] At some electrode positions, discharges from one individual unit could have a frequency as high as 40/sec although no signs of activity from other unit could be discerned

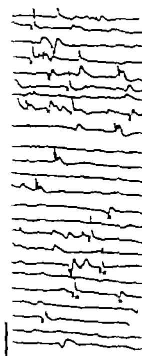


Fig 1 Consecutive sweeps f typical electromyographic ctivity recorded from huma vocal muscle during quiet breathing. Baseline fairly unstabl In the first ight tracings in the following the activity has declined, three different motor unit potential (*a*, *b*) being clearly discerned. Horizontal ba 5 msec, ertical bar 1 mV

Not infrequently the electrode could be kept in a sufficiently stable position to permit recordings from single motor units during repeated respira tory phases. It seems pertinent briefly to describe the typical pattern of these discharges since previous studies in man seem to have yielded contradictory results (cf Faaborg Andersen 195 Hiroto *et al.*, 1968, and Discussion below)

In some units the discharge frequency was comparatively stable and entirely independent of the respiratory phases. In other units the frequency varied with respiration some of these units discharged only during one of the respiratory phases whereas others fired continuously modulating their frequency with respiration. There were large variations in the number of cyclically active units in different subjects. As a rule respiratory units were observed at a comparatively small number of recording points, and only one or two such units were found at each point, but in a few subjects a large number of units of this type were encountered at several different electrode positions. It seems unlikely that these individual variations should have been due to differences in electrode positions, since the number of potentials discharging cyclically with respiration was about the same in both vocal muscles in all the bilateral experiment performed.

There were also indi idual ariations as regards the respiratory phase in

which the units appeared In about one-third of the subjects only inspiratory units were encountered, in one-third only expiratory units in the remaining third both inspiratory and expiratory units occurred in the same muscle Such individual variations should not have been due to accidental insertion of the recording electrode in nearby muscles, since the cyclically active units invariably discharged in the same respiratory phase in the muscles on both sides and occasionally both inspiratory and expiratory units could be en countered at one and the same electrode position

Discharge pattern during deglutition

Since there is a rather regular and marked increase in the activity of the intrinsic laryngeal adductor muscles during deglutition (cf Faaborg Ander sen, 1957) this type of activation was employed to study the discharge pattern during a contraction of high intensity in the vocal muscles To reduce the risk of injury caused by the needle tip only three recordings were made from each muscle during deglutitions, and hence it was not possible to decide with full certainty to what extent the recordings were reproducible or were dependent on the electrode site However the recordings always displayed a pronounced interference of discharges from different motor units of the type observed on maximal contraction of the extremity muscles. The maximal amplitude of this interference pattern was usually in the range 1.5–2.5 mV but might vary considerably at different points in the same muscle In one extreme case it varied between 0.8 and 4 0 mV

Shape duration and amplitude of the action potentials

In all 400 action potentials from 18 muscles were identified in the 13 subjects examined Most of the potentials were di or triphasic, in agreement with observations made in previous investigations (Faaborg-Andersen, 1957) but a fairly large number had other wave forms Thus, 4% were monophasic 12 tetraphasic and 5% had more than four phases In the latter group, classed as polyphasic no potential had more than seven phases. The tracings of the potentials were usually smooth but part of them displayed some degree of notching in one or more of their phases, as has been noticed also in other muscles (cf Buchthal & Clemmesen 1941 Petersén & Kugelberg 1949) Obviously the appearance of such notches will depend on the sweep speed and amplification used In the present study they were observed in about 15% of the potentials.

The incidence of potentials of a certain phasicity varied widely in the different muscles, as would be expected in view of the limited number of potentials recorded from each muscle Thus, e.g., polyphasic potentials had an incidence ranging from 0 to 15% and this latter percentage was observed in no less than 3 out of the 18 muscles examined. Tetraphasic action potentials, by some authors referred to the polyphasic group, had an incidence varying from 0 up to 25

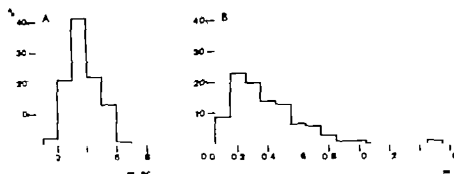


Fig. 2. Histogram to show duration and amplitude of action potentials recorded from normal vocal muscles. *A*, distribution of durations of 460 potentials from 18 muscles. *B*, amplitude distribution of 323 potentials from 14 muscles.

Most of the potentials had a duration of 3 to 6 msec (Fig. 2 *A*) but about 25% were briefer or longer in duration, the lowest value found being 1.3 msec and the highest 7.5 msec. The distribution of the potential durations differed considerably, thus, e.g. in some muscles 10–15% of the potentials had durations below 2 msec, whereas in the other muscles no potentials were briefer than 2 msec. The mean potential duration in single muscles ranged from 3.03 ± 0.94 msec (S.D., 20 potentials) to 4.43 ± 0.84 msec (27 potentials) as against a mean value of 3.76 ± 1.01 msec for the total number of potentials.

To some extent, the age distribution of the subjects under study might be responsible for these variations (cf. Petersén & Kugelberg 1949, Sacco *et al.* 1962). The average potential durations in two age groups, viz. subjects aged 20–30 and 40–60 respectively, appear from Table 1. The mean duration was found to be 0.8 msec longer in the older group which is a statistically significant difference. Also within the two age groups there were wide variations in the mean action potential durations of different muscles (cf. last right-hand column in Table 1), but these variations may be due to errors of random sampling.

The amplitude was measured in 323 motor unit potentials from 14 muscles in 10 subjects. As appears from Fig. 2 *B* the distribution is skew, most of the potentials having amplitudes around 0.2 mV as against a mean of 0.41 mV. In the different muscles the mean amplitude varied over a very wide

TABLE 1. *Duration of motor unit potentials in two age groups*

Age (years)	No. of muscles	No. of potentials	mean, total (msec)	s. (msec)	s. (msec)	Range of means for single muscle (msec)
20–30	9	197	3.43	1.03	0.07	3.03–3.83
40–60	7	146	4.21	1.00	0.08	3.60–4.44

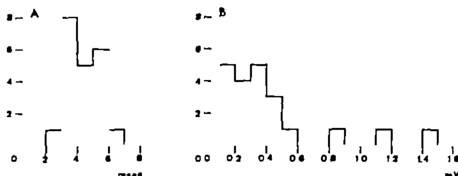


Fig 3 Distrib tion f duration (*A*) a d mplit des (*B*) of 21 polyphasic potentials recorded from normal vocal m scles. Ordinat : number f potentials.

range the lowest and highest sample means were 0.23 ± 0 13 mV (30 potentials) and 0 60 ± 0.28 mV (23 potentials) respectively Hence it seemed impractical to use mean values as a basis for determinations of amplitude deviations in pathological cases. Consequently the 10th and the 90th percentiles of the total amplitude distribution were calculated and found to be 0 15 and 0 70 mV respectively hence 80 % of the potentials had amplitudes over this range In 9 of the 14 muscles tested more than 80 % and in the remaining 5 muscles 75–80 % of the potentials had amplitudes ranging between 0 15 and 0 70 mV This range thus seems to give a fairly good estimate of the normal amplitude distribution

Separate determinations were made of the variations in duration and amplitude of the polyphasic potentials since these parameters should be useful in the analyses of pathological changes in the electromyographic activity The duration of all polyphasic potentials identified ranged from 2.0 to 6.2 msec and as appears from Fig 3 *A* the duration distribution of these potentials *agrees well with that depicted in Fig 2 A for the total number of* potentials measured. In this group of polyphasic potentials the amplitudes varied between 0 1 and 1.5 mV and as shown in Fig 3 *B* the amplitude distribution shows a fairly good correlation with the values found for he total number of potentials, as presented in Fig. 2 *B*

Since it may be difficult to distinguish fibrillation potentials from the

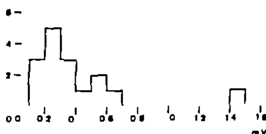


Fig 4 Amplit d distributio f potential f durati n of 2 msec or less encount red in n rmal ocal muscles. Ordinat umber f potentials.

action potentials of short duration observed in normal muscles, the ampli tude distribution of all potentials of a duration equal to or less than 2 msec was determined As appears from Fig 4 the amplitude variation was fairly similar to that of the total number of potentials. Also their wave forms showed the same variations as the other potentials. These findings should be of value for the identification of fibrillation potentials in paretic muscles.

DISCUSSION

Many of the motor units encountered in the present investigation discharged at a frequency of about 40/sec which is a high rate considering the care taken to achieve relaxation At such high discharge frequencies in the extremity muscles there is a considerable voluntary contraction effort and discharges can usually be recorded from several simultaneously active motor units. The resting activity in the vocal muscle is not under voluntary control and the contraction developed cannot readily be estimated, but certain findings suggest that it is moderate Thus, at a frequency as high as 40/sec often only one or a few units were active, as checked at many electrode sites. Besides, the twitch duration in the vocal cord can be expected to be very short since it has been found in studies of a number of different animal species that the twitch duration of certain vocal cord adductors, among them the vocal muscle is as brief as 30–40 msec (Mårtensson & Skoglund, 1064) If similar values apply to the vocal muscle in man, the degree of summation of the motor unit twitches is not likely to be high even at the discharge frequencies observed

It cannot be excluded that the indi idual variations in the respiratory pattern of the motor unit discharges observed in the present investigation are, in part, due to differences in the anesthesia of the mucosa, since it has been found in animal experiments that the afferent discharges from the mucosa have a very pronounced effect on the motoneurons of the intrinsic laryngeal muscles (Eyzaguirre & Taylor 1963) In the cat, respiratory move ments of the larynx as well as jets of air blown onto the vocal cord consti tute stimuli capable of eliciting afferent discharges from superficially situat ed touch receptors (Sampson & Eyzaguirre 1964) and gentle probing of the mucosa was shown to elicit a reflex inhibition of inspiratory discharge and an increase of the expiratory discharges in filaments of the recurrent laryngeal nerve If similar mechanisms are operative in man and if the respiratory movements and the air stream passing through the larynx constitute stimuli adequate to activate such receptors in the mucosa, then administration of a local anesth tic would result in a reduction of the afferent inflow and consequently a lowering both of the reflex activation of expiratory and the inhibition of inspiratory units.

The experiments on cat performed by Eyzaguirre & Taylor (1963) indi cated that the effects on the respiratory discharges are more readily elicited from the supragl ttic than from the ubgl ttic part of the mucosa If this

applies also to man the site of application of the local anesthetic may be an important factor influencing the discharge pattern of the vocal muscle In the experiments reported by Faaborg Andersen (1957) the activity in the vocal muscle always increased with inspiration In his experiments, the supraglottic part of the laryngeal mucosa was anesthetized and this may have deprived expiratory units of the naturally occurring excitatory afferent impulse flow from this part of the mucosa Hiroto et al (1968) claimed that no activity is present in the normal thyroarytenoid muscles during the inspiratory phase. It does not appear from their report whether or not the laryngeal mucosa was anesthetized in their experiments. Since they considered the presence of inspiratory activity as evidence of a misdirected sprouting in cases classified as neurogenic pareses, information on the anesthesia used and its site of application would be of interest

In accordance with previous observations (Weddell *et al.*, 1944) the duration of the motor unit potentials was comparatively short Means and dispersions as found in the present investigation correspond reasonably well to those reported in studies using bipolar electrodes (Faaborg-Andersen, 1957) When using such electrodes, the motor unit potentials recorded in extremity muscles are far briefer than those recorded with concentric electrodes of the type employed in the present study (Petersén & Kugelberg, 1949 Buchthal *et al.*, 1954) Similar differences in potential duration with the type of electrode used do not seem to occur in recordings from the vocal muscle It seems probable that this is due to the organization of the muscle fibers within the motor units and to the small inter-space variation between recording point and end plates in a muscle of such a small size as the vocal muscle

Owing to the continual movements of the vocal cords, repeated minute changes in electrode position relative to the muscle fibers in the course of the recordings cannot be ruled out, and hence some of the potentials recorded may have been provoked by mechanical irritation of muscle fibers or nerve branches. From studies of other muscles it is known that this type of stimulation usually results in action potentials as brief as fibrillation potentials (Kugelberg & Petersén 1949) and thus of the same duration as the shortest potentials observed in normal vocal muscle Whether these brief potentials are set up by motor units or represent insertion activity cannot be decided with full certainty The concept that they are motor unit discharges gains support from the fact that they were never observed in bursts of high frequency discharges, which are characteristic of although not invariably seen in insertion activity Besides, the amplitudes of these short potentials as well as their wave forms varied in a way closely similar to that observed in the total number of potentials

The presence of polyphasic potentials or di or triphasic waves of exceptionally large amplitude, though apparently less than 1 mV has been regarded as proof of a reinnervation of paretic vocal muscle (Haguenauer *et al* 1967 Hiroto *et al* 1968) As is evident from the present investigation, a fairly large proportion of such potentials may however occur in the normal

muscle and hence potentials of these types cannot be considered as evidence of a pathologic process unless they occur in a significantly increased proportion or deviate significantly from the normally occurring potentials in shape or amplitude.

ZUSAMMENFASSUNG

Unter Verwendung eines perkutanen Verfahrens, welches vorher in retrograden Inspektionen des Kehlkopfes benutzt w rden ist, haben wir eine Methode für elektromyographische Untersuchungen des menschlichen Musculus vocalis ent wickelt, wodurch es in den meisten Fällen möglich ist Potentiale von etwa zwanzig verschiedenen motorischen Einheiten in demselben Muskel zu identifizieren und welche für den Patienten kaum schmerzhaft ist. Es wird über die Befunde bei Ableitungen von achtzehn normalen Muskeln bei acht gesunden Versuchspersonen und fünf Patienten mit einseitiger Stimmbandparese berichtet.

Bei ruhiger Atmung war in mehreren motorischen Einheiten ine kontinuierliche Aktivität vorhanden Die Entladungsfrequen war in einigen Einheiten atmungsabhängig diese Gruppe enthielt sowohl expirationsaktive wie inspirationsaktive Einheiten.

Di Potentiale der motorischen Einheiten konnten bis zu sieben Phasen enthalten. Die Mehrzahl der Potentiale waren di- oder triphasisch fünf Prozent waren polyphasisch. Die mittlere Dauer der Potentiale war 3 70 ± 1 01 Millisek (n = 469) Ein kleiner aber doch signifikanter Unterschied in den Mittelwerten der Potentialdauer liess sich zwischen zwei Altersgruppen, 20–30 bezw 40–60 Jahre alt, nachweisen. Die mittlere Amplitude all Ei zelpotentiale war 0 41 mV (n = 323) das Perzentil (0 8) lag zwischen 0 1 und 0 70 mV Während Schlucken wechselte die Maximalamplitude des Interferenzmusters in den meisten Fällen zwischen 1,5 und 2,5 mV

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AN ABNORMAL SPEECH PATTERN ASSOCIATED WITH AN OROFACIAL ANOMALY

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The speech of a 24-year old white female with multiple orofacial anomalies was studied by articulations testing and connected speech samples. Speech physiological characteristics were investigated by using cinefluorographic analysis. The results of these studies, as well as the unique pattern of compensatory articulatory movements were reviewed.

Many authors (Blyth, 1959 Fymbo, 1936 Greene, 1937 Marge, 1965 Palmer 1948 Subtelny & Subtelny 1962) have noted that oral anomalies can have definite adverse effects on speech production and speech intelligibility The present case is reported because of the unique articulatory patterns developed in order to help compensate for the limitations presented by the orofacial anomalies.

CASE REPORT

This 24-year-old white female initially had a congenital cleft lip and palate and ectodermal dysplasia. There was also an apparent mandibular prognathic condition according to the clinical and radiographic analysis. Permanent teeth were congenitally missing in the maxillary incisor and cuspid areas and in the mandibular incisor right cuspid, and right bicuspid areas. Severe underdevelopment of alveolar bone was noted in areas where the teeth were congenitally missing In the closed centric position the space from the mandibular alveolar ridge to the maxillary alveolar ridge in the anterior region measured approx 40 mm.

The mandible was set back approx. 10 mm employing a vertical ramusotomy procedure, to accomplish a more favorable anteroposterior relationship of the maxilla to the mandible (Figs. 1 and 2) The setback procedure provided a more favorable foundation for the dentures and a better position of the lips and soft tissue structures. The remaining teeth were either extracted or restored and removable partial dentures were provided (Fig 3)

Cinefluorographic analysis revealed reduced palatal mobility resulting in a velopharyngeal gap during sustained phonation and connected speech. Subjective description of speech included evident nasality and multiple misarticulations due primarily to faulty tongue placement



Fig 1 Alveol ridge relationship bef re rgical setback of the mandibl

The upper lip was very short and only slightly mobile The same tight ness was not observed on either side of the lip or in the lower lip It was noted that the relaxed mouth position for the patient was a slightly open position probably contributing to the patient s tendency to mouth breathe

Detailed articulatory evaluation using the Diagnostic Form of the *Templin–Darley Test of Articulation* (1960) revealed 70 errors out of the 176 items tested, indicating poor speech

It must be remembered that the articulation inventory reported above was taken from samples of single words. Careful analysis of articulatory patterns in connected speech samples and in isolated consonant vowel contacts revealed further information as to articulatory patterns.

Production of the bilabial sounds was accomplished through a compensa



Fig 2 Alveolar ridge rel tion hip fter surgical setback f the mandibl



Fig 3 Remov bl partial dent re in place

tory movement of the tongue since the patient reported it was an extra effort to touch the lips together in the usual manner of production. Sufficient intraoral pressure was not built up in this procedure for correct production of these phonemes, and three speech pathologists independently scored them as distorted

Figs. 4 and 5 show the abnormal movement of the tongue to produce the [p] and [b] sounds. Instead of the usual lip movement for these sounds the tongue was protruded between the lips and the sounds were distorted. This position was also used to produce [s] and [m]. Acoustically the bilabial sounds were closer to lingual-alveolar productions such as [t] or [d] even though the tongue approximated a [θ] production position This held true for bilabial productions in the initial and medial positions of words. In the final position [p] and [b] were omitted when heard without a visual cue, but were actually produced in the compensatory fashion when manner of production alon was scored. It was interesting to further note that the tongue was not elevated for production of the [t] in the initial and medial positions. Instead, an [h] was substituted. This, however was not a free-flowing" [h] but was accomplished by some degree of glottal constriction that approximated a build-up of intraoral pressure and assisted in making the [h] substitution acoustically more like the [t] being attempted. Production of [k] were ac



Fig 1 Photograph illustrating faulty (P) production

complished in the same manner as the [t] described above in the initial and medial positions, and the glottal [h] was substituted in the final position. This patient did not elevate the tongue for the [l] production. It remained just below the lower central incisors, and thus, acoustically it was not perceived as misarticulated Acoustically [ʃ] was scored as omitted independently by two speech pathologists. Although it was noted that in production the [ʃ] was accomplished in a [θ] position it was interesting that [θ] productions were scored omitted and distorted

DISCUSSION

Bloomer (1957) has said that the most significant factor in consonant production is the locus of the articulatory valve Clearly in this case report faulty articulation was due to inappropriate articulatory patterns which produced consonant distortion

The speech and agility of this tongue movement was amazing Where other speakers might have considered these extraneous movements, the patient felt they were easier to achieve than touching the lips together and the movements were incorporated into connected speech without affecting speech tempo



Fig 5 Photograph illustrating f lty (*B*) production

A plastic surgery procedure designed to lengthen this patient s upper lip is planned after which speech therapy will be used to attempt to assist her in using her lips appropriately to achieve acoustically correct bilabial consonant production.

ZUSAMMENFASSUNG

Die Sprechwei einer 24jährigen wei sen Patientin mit l lf chen oral-f cialen Anomalien wurde Hand von Artik lationstest u d zusammenhängenden Sprachproben u tersucht Sprachpb si logische Charakteristika wurden mit Hilfe von cinefluorographischer An lyse untersu l t. Di Resultat dieser Studien al auch das inzigartige Schema on kompensatorischen Art k lationsbewegungen wurden nalysiert.

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DIE OSSIFIKATION DES MENSCHLICHEN KEHLKOPFES AUS PHONIATRISCHER SICHT

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Die Untersuchungen zur Ossifikation des menschlichen Kehlkopfes wurden unter dem Gesichtspunkt der Akzeleration der Jugendlichen und zur Erfassung eines Teilaspektes beim Alternsvorgang der menschlichen Stimme durchgeführt. Es wurden 450 seitliche Kehlkopfröntgenaufnahmen von 250 männlichen und 200 weiblichen kehlkopfgesunden Patienten ausgewertet. Erste Ossifikationszeichen fanden sich bei weiblichen Patienten im 15. Lebensjahr. Für den Mann wurde der Ossifikationsbeginn bisher übereinstimmend für das 18.–20. Lebensjahr angegeben. Wir konnten dagegen bereits erste Knochenkerne im 16. Lebensjahr nachweisen und werteten ausgedehnte Verknöcherungen bei Patienten unterhalb des 18. Lebensjahres mit der gebotenen Zurückhaltung als Wachstumsakzelerationserscheinung. Weiterhin stellt die Kehlkopfknorpelossifikation einen wesentlichen Faktor beim Alternsvorgang der menschlichen Stimme dar.

Wir haben Untersuchungen zur Ossifikation des menschlichen Kehlkopfes besonders unter zwei Gesichtspunkten durchgeführt. Es sollte dabei geklärt werden, ob durch die Wachstumsakzeleration der Jugendlichen eine frühere Verknöcherung des Kehlkopfskeletts einsetzt, und andererseits wollten wir einen Teilaspekt beim Alternsvorgang der menschlichen Stimme erfassen.

Es wurden 450 seitliche Kehlkopfröntgenaufnahmen von kehlkopfgesunden Patienten ausgewertet. Davon entfielen 250 Aufnahmen auf männliche Patienten im Alter zwischen 13 und 84 Jahren und 200 Aufnahmen auf weibliche Patienten im Alter zwischen 13 und 75 Jahren.

Folgende Befunde, die nach Geschlecht und Lebensdezennien aufgeschlüsselt wurden, konnten dabei erhoben werden.

Bei *männlichen Patienten im zweiten Lebensdezennium* fanden sich die ersten Ossifikationszeichen bei einem Patienten im Alter von 15 Jahren in Form eines kleinen Kalkkerns im hinteren unteren Schildknorpelrand am Übergang zum Unterhorn. Von diesem Kalkkern aus schreitet der Ossifikationsvorgang nach oben im hinteren Schildknorpelrand und nach vorn im unteren Schildknorpelrand fort. Beginn und späterer Verlauf der Ossifikation sind aber individuell sehr unterschiedlich (Thost 1913). So waren auch bei vielen unserer männlichen Patienten erste Knochenkerne erst zwischen dem 18. und 20. Lebensjahr nachweisbar.

Es fanden sich aber beispielsweise bei einem 17jährigen bereits eine recht





Abb. 1. M ... ko ... J hre. Abb. ... Mä ... licher Kehlkopf — 3. Jahre.

aus, dehnt ... t ... n B reich des Schildknorpelhinter und Unterrandes, d ... Rin ... r ... pelp latte und angedeutet auch des Oberrandes des Ring ... w e bei einem 19jährigen bereits die Anlage eines im ... tu d der Schildknorpelplatte gelegenen Ossifikation kern ... n ... Ringknorpels setzt kurz nach Auftreten des ersten K ... ildknorpel von einem horizontal gelegenen Knoch ... i ... r Gelenkfläche für den Stellknorpel aus ein. Dieser e ... mal besonders aber bei isolierter Verknöcherung im ... ben Jahrzehnt als Hypopharynxfremdkörper da er ... schatten des Hypopharynxraumes projiziert wird. Mit ...) weitere Ringknorpelossifikation erfolgt von einem am V ... u ... ildknorpelgelenkfläche sich entwickelnden Knochenkern.

Im *dritten Lel ... t ... nt* setzt sich die Ossifikation vom hinteren unteren Schildknorpelr n ... u Hinterrand nach oben und im Unterrand nach vorn fort. Bei d r M ... rzahl der Patienten ist der Schildknorpelhinterrand vollständig os ... ifiz ... rt, w ... ei die O ... sifikation des Oberhornes in der zweiten Hälfte des dritten D ... nnium einsetzt. Gleichzeitig ist die Ossifikation des Unterrandes bis über di ... halbe Breite vorgerückt. Die Anlage des vorderen unteren im Schildkn ... rpelwinkel gelegenen Kern ... fand sich bei 50 ... der Patienten. Vom 27. Leben jahr aufwärts setzt dann auch die Ossifikation im vorderen oberen Schildknorpelwinkel ein, und vereinzelt fand sich auch schon ein Kern in der Mitte der Schildknorpelplatte. Die Ossifikation des



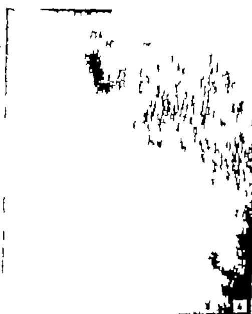


Abb. 3. Männlicher Kehlkopf — 57 Jahre *Abb.* 4. W. [illegible] K[illegible]hlkopf 16 J[illegible]

Ringknorpels ist bereits relativ weit fortgeschritten. Dabei ist bemerkenswert, dass sie häufig nur die oberen und ventralen Teile der Ringknorpelplatte betrifft, während die Dorsalfläche knorpelig bleibt.

Im Ringknorpelbogen schreitet die Ossifikation vom Oberrand nach unten





Abb. 5. Weibl[illegible]her Kehlkopf 37 Jahre *Abb.* 6. W[illegible] K[illegible]hlkopf — 62 J[illegible]

und vorn fort, wobei der Unterrand in diesem Alter fast nie eine Ossifikation zeigte. Die Ausdehnung der Kehlkopfossifikation kann auch in diesem Alter sehr unterschiedlich ausgeprägt sein.

Die Ossifikationsvorgänge im *vierten Lebensdezennium* sind durch die Verknöcherung des Schildknorpelvorderrandes mit Auftreten des schon beschriebenen Knochenkerns im vorderen oberen Schildknorpelrand sowie *einer Verknöcherungszone vom Tuberculum thyreoideum caudale schräg* nach oben zum oberen Schildknorpelrand mit gleichzeitigem Auftreten eines Knochenkerns in der Schildknorpelplattenmitte gekennzeichnet. Die Ringknorpelossifikation verläuft in der schon beschriebenen Weise weiter ohne Besonderheiten aufzuweisen.

Im *fünften Lebensdezennium* dehnt sich die Ossifikation von allen Verknöcherungszonen zur Schildknorpelplattenmitte hin aus. Es findet sich jetzt auch eine Ossifikation des Schildknorpeloberrandes, welche aber auch völlig fehlen kann. Im Ringknorpel wird in diesem Alter relativ häufig, neben dem schon beschriebenen horizontalen oberen Knochenkern, eine strichförmige vertikale Verknöcherungszone im dorsalen Teil der Ringknorpelplatte sichtbar, die gleichfalls zur Verwechselung mit Hypopharynxfremdkörpern Anlass geben kann (Minnigerode 1959). Auch in diesem Alter bleiben aber häufig der dorsale Teil der Ringknorpelplatte und der Unterrand des Schildknorpelbogens knorpelig.

Im *höheren Lebensalter* finden sich lediglich graduelle Unterschiede in der Ausprägung der Ossifikation. In den meisten Fällen ist die Schildknorpelplatte bis auf zwei Knorpelinseln ventral und dorsal der schrägen Verknöcherungszone vom Tuberculum thyreoideum caudale zum oberen Schildknorpelrand ossifiziert. Auffällig ist lediglich, dass relativ häufig die Verknöcherung des Schildknorpeloberrandes und die Ossifikation des unteren und dorsalen Teils der Ringknorpelplatte sowie des Unterrandes des Ringknorpelbogens unvollständig bleiben.

Bei *weiblichen Patienten im zweiten Lebensdezennium* fanden sich die ersten Ossifikationszeichen vom 15. Lebensjahr ab gleichfalls als kleine Kerne im unteren hinteren Schildknorpelrand. Die Ossifikation schreitet, *wie beim männlichen Geschlecht, im hinteren Schildknorpelrand* nach oben und geringer im unteren Rand nach vorn fort. Die Ossifikation des Ringknorpels beginnt, wie beim Mann, von dem oberen horizontal gelegenen Knochenkern aus und zeigt im Verlauf keine wesentlichen Unterschiede zu der des Mannes, wie wir in Übereinstimmung mit Fraenkel (1908), Lanz & Wachsmuth (1955) sowie Scheier (1902) feststellen konnten. Einen wesentlich späteren Beginn der Ossifikation im Ringknorpel, wie ihn Sonnenkalb (1914) (Anfang des dritten Jahrzehnts) beschreibt, konnten wir nicht feststellen.

Bei den von uns untersuchten Patientinnen im Alter unter 20 Jahren war die Ossifikation im Vergleich mit gleichaltrigen Männern häufig stärker ausgeprägt. Zwei Patientinnen im Alter von 20 Jahren zeigten bereits recht erhebliche Ossifikationen im Bereich des Schild- und Ringknorpels, sogar

schon mit Anlage eines Knochenkernes im vorderen oberen Schildknorpelwinkel.

Im *dritten Lebensjahrzehnt* entwickelt sich die Ossifikation der Kehlkopfknorpel der Frau weiterhin ähnlich wie die des Mannes, besonders im Schildknorpelunter- und Hinterrand. Die Ossifikation im Unterrand geht dabei nicht über die mittlere Breite hinaus. Ein gewisser Geschlechtsunterschied besteht nur darin, dass bei der Frau vom Schildknorpelhinterrand parallel zur Unterkante ausläuferförmige Verknöcherungen nach vorn auftreten können. Bei vier Patientinnen im Alter von 29 Jahren konnte bereits ein median im Schildknorpelwinkel gelegener Knochenkern nachgewiesen werden. Die Ossifikation des Ringknorpels entspricht im wesentlichen der des Mannes.

Ab viertem Lebensdezennium kann neben Verknöcherungen wie beim männlichen Geschlecht ein mehr weiblicher Verknöcherungsmodus zur Ausprägung gelangen. Dabei erfolgt die Ossifikation entweder in ganzer Breite vom hinteren Schildknorpelrand nach vorn (Scheier 1902) oder es finden sich ausläuferförmige Ossifikationszonen vom hinteren Schildknorpelrand parallel zur Unterkante, wobei die Ausdehnung nach vorn von unten nach oben terrassenförmig abnimmt (Fraenkel, 1908).

Wir konnten jedoch bereits bei 30% der Patientinnen einen im Schildknorpelwinkel gelegenen Knochenkern nachweisen. Die Ausdehnung der Kehlkopfknorpelossifikation ist in diesem Alter bei der Frau deutlich geringer als beim Mann. Dieser oben beschriebene weibliche Verknöcherungsmodus ist in den *höheren Lebensjahren* stärker ausgeprägt und fällt besonders dadurch auf, dass das vordere Schildknorpeldrittel bis auf den im Schildknorpelwinkel gelegenen Kern bei vielen Frauen auch im höheren Alter knorpelig bleibt.

Im Gegensatz zu Griebel (1952), Lanz & Wachsmuth (1955), Mittermaier (1952), Scheier (1902), Waldapfel (1938) und Wustrow (1963), welche bei Frauen *einen im Schildknorpelwinkel gelegenen Knochenkern nur sehr* selten im höchsten Alter und fast nie in Verbindung zur Ossifikationszone im Schildknorpelunterrand feststellen konnten, fanden wir diesen noch häufiger als Chievitz (1882) und Fraenkel (1908) bei fast allen von uns untersuchten Patientinnen oberhalb des 48. Lebensjahres. Dieser Kern ist also nicht typisch für das männliche Geschlecht. Oberhalb des 54. Lebensjahres war auch fast regelmässig eine Verbindung dieses Kernes mit der Verknöcherungszone im unteren Schildknorpelrand nachweisbar. Gleichzeitig können auch bei Frauen in Übereinstimmung mit Fraenkel Knochenzapfen vom unteren Rand des Schildknorpels nach oben auftreten. Die Betonung dieser Befunde erscheint in Bezug auf die Altersveränderungen der weiblichen Stimme besonders wichtig.

Zur Ossifikation der Giessbeckenknorpel kann keine Aussage getroffen werden, da diese auf den verwendeten seitlichen Kehlkopfaufnahmen durch Überlagerung nicht eindeutig zu beurteilen sind. Die elastischen — oder Netzknorpel des Kehlkopfes verknöchern auf Grund ihrer Struktur praktisch nicht.

Die Wachstumsakzeleration und ihre Beziehungen zur Kehlkopfknorpelossifikation

Es kann heute als gesichert gelten, dass die Ossifikationsvorgänge im Kehlkopf in einem engen Zusammenhang zur Pubertät auftreten und endokrinologische Faktoren einen bedeutenden Einfluss auf diesen physiologischen Prozess haben Erste Knochenkerne treten nach Abschluss der Pubertät auf und so konnten wir erste Kalkeinlagerungen bei einem männlichen Patienten bereits im 16 Lebensjahr feststellen und bei mehreren weiblichen Patientinnen waren im 15 und 16 Lebensjahr gleichfalls deutliche Ossifikationskerne sichtbar

Chievitz (1882) Fraenkel (1908) Lanz & Wachsmuth (1955) Sonnenkalb (1914) und Wustrow (1963) geben den Ossifikationsbeginn für das männliche Geschlecht übereinstimmend mit 18–20 Jahren an während Scheier (1902) einen früheren Beginn vermutete da er schon mit 19 Jahren ausgedehntere Ossifikationszonen fand Für das weibliche Geschlecht wird der Ossifikationsbeginn von Fraenkel und Sonnenkalb mit 14 bzw 15 Jahren angegeben, während Lanz & Wachsmuth sowie Wustrow erste Ossifikationszeichen erst mit 22 Jahren beschreiben Wie wir bereits betonten fanden wir bei einem männlichen Patienten erste Ossifikationsherde mit 15 Jahren, jedoch in den meisten Fällen erst zwischen dem 18 und 20 Lebensjahr während bei weiblichen Patienten die Ossifikationen zwischen dem 15 und 20 Lebensjahr durchaus häufiger vorhanden und auch stärker ausgeprägt waren Im Vergleich mit den Ergebnissen von Fraenkel (1908) der gleichfalls bei einem Mädchen erste Ossifikationsherde im 15 Lebensjahr fand liess sich bei unseren jetzigen Untersuchungen keine frühere Kehlkopfossifikation bei weiblichen Patienten nachweisen Wir glauben jedoch annehmen zu können dass es sich bei den männlichen Patienten, bei welchen wir bereits in jüngeren Jahren stärkere Ossifikationszeichen nachweisen konnten, unter gleichzeitiger Berücksichtigung der *gesamtkörperlichen Entwicklung dieser Patienten, um Zeichen der* Wachstumsakzeleration handelt die sich in diesen Fällen auch in der Ausdehnung der Kehlkopfossifikation wiederspiegelt

Die Beziehung zwischen Kehlkopfossifikation und Altersstimme

Die Entstehung der Altersstimme hängt mit zahlreichen biologischen Faktoren zusammen die die Altersinvolution des Gesamtorganismus sowie spezielle degenerative biochemische und endokrinologische Veränderungen im Bereich des Windkessels, des Tongenerators und des Ansatzrohres betreffen Zu diesen Problemen hat in letzter Zeit besonders Böhme (1969) Stellung genommen Hier soll nur auf die Probleme eingegangen werden die sich aus der physiologischen Kehlkopfknorpelossifikation bei der Entstehung der Erwachsenen und Altersstimme ergeben

Wir haben bei ungefähr der Hälfte der von uns untersuchten Patienten gleichzeitig den Stimmklang beurteilt sowie die Sprechstimmlage und den

Stimmumfang bestimmt Dabei konnten keine Beziehungen zwischen dem Grad der Kehlkopfossifikation und der Sprechstimmlage oder dem Stimm umfang festgestellt werden Es fanden sich lediglich die bekannten Altersveränderungen mit Einschränkung des Stimmumfanges, einer Erhöhung der Sprechstimmlage bei Greisen und einer Senkung derselben bei Greisinnen.

Diese Ergebnisse verdeutlichen, dass es sich bei der Entstehung der Altersstimme um einen komplexen biologischen Vorgang handelt, der von vielen Faktoren beeinflusst wird. Dagegen liessen sich aber Beziehungen zwischen der Ausdehnung der Kehlkopfknorpelossifikation und dem Stimm klang finden. Da es sich dabei jedoch um rein subjektive Beurteilungen handelt, müssen sie mit der hierbei gebotenen Zurückhaltung betrachtet werden.—Diese Befunde fielen uns bei jüngeren Patienten auf die im Vergleich mit der durchschnittlichen Ossifikation der Kehlkopfknorpel bei Gleichaltrigen bedeutend stärkere Grade derselben aufwiesen. Diese Patien ten hatten häufig einen Stimmklang, der einem höheren Alter als ihrem kalendarischen entsprochen hätte. Besonders auffällig war dies bei zwei 20jährigen Patientinnen mit erheblich fortgeschrittener Ossifikation des Schild- und Ringknorpels. Dabei wiesen diese Patienten jedoch keinerlei Zeichen einer Stimmerkrankung auf Bei ausgesprochen gering ausgeprägter Kehlkopfossifikation und ansonsten normaler gesamtkörperlicher Entwick lung waren keine Änderungen des Stimmklanges zu bemerken.

Nach Luchsinger (1959) muss der Kehlkopf als Vibrationssystem betrach tet werden welches durch einen grossen Dämpfungsfaktor ausgezeichnet ist Er bildet den untersten Abschnitt eines Systems von gekoppelten Resonatoren, welche auf die Schallquelle reagieren. Die physiologische Ossifikation der Kehlkopfknorpel muss im System des Geräuschgenerators zu einer Ände rung des Dämpfungsfaktors führen. Dadurch werden wiederum Verschie bungen im angekoppelten Resonatorensystem ausgelöst, die im Endeffekt im Zusammenwirken aller Faktoren bei der Entstehung der Erwachsenen und Alters timme, zu iner Änderung des Stimmklanges führen.

Weiterhin muss die verminderte Elastizität der Kehlkopfknorpel und damit des gesamten Kehlkopfes Berücksichtigung finden. Sie ist besonders wichtig für die Klangfarbe der Stimme So konnte Luchsinger (1950) beim gedeckt gesungenen Ton ein Auseinanderweichen der Schildknorpelplatten nachweisen Diese Möglichkeit entfällt natürlich mit zun hmendem Alter und somit werden auch die Möglichkeiten des älteren Menschen zur Ände rung der Stimmklangfarbe eingeschränkt Dies gilt auch für Frauen ober halb des 50 Lebensjahr s, da bei ihnen gleichfall eine Ossifikati n im Schildknorpelwinkel eintritt die ein Auseinanderweichen der Schildknorpel platten i ht m hr zulässt

Die Tatsache der O sifikation allein rlaubt k ine Rückschlüsse auf das biologisch oder kalendarische Alter der Patienten da sie physiologisch in weiten (nzen Schwankungen unt rworfen ist Bei Inbetrachtziehung aller beim Alt n prozess der m nschlichen Stimm mitwirkenden Faktoren spielt ie abei icher eine wesentlich Rolle

Die beigefügten Abbildungen sollen die beschriebenen Befunde in den ein zelnen Lebensdezennien bei männlichen und weiblichen Patienten ergänzen und die Unterschiede im Ossifikationsverlauf verdeutlichen

SUMMARY

Examinations as to the ossification of the human larynx were performed by paying regard to the aspect of acceleration of juveniles, and in order to get hold of a part aspect in the growing old of the human voice 450 lateral larynx X-ray photos of 250 male and 00 female patients with sound larynx were evaluated. First signs of ossification were found in female patients aged 15. Up to now the beginning of ossification for men was in accordance told to be the age of 18 to 20. We were able however to show first bone-nuclei at the age of 16, and valued extensive ossification with patients below 18 with necessary reserve as a symptom for the increase of acceleration Besides the ossification of the larynx cartilage is an important factor in the growing old of the human voice

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MALIGNANT TUMORS OF HEAD AND NECK IN CHILDHOOD

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The frequency of primary malign nt tumors of the head and neck in childhood in a defined population is given. The need of earl diagnosis is stressed. Fine needle aspiration biopsy is a valuable procedure. Ex t ded surgery should, if possible be carried out without delay and, if needed, be followed by prompt and dequate radiotherapeuti measures. The prognosis does not seem to be so pessimisti as is usually believed. An intense follow-up program and prophylaxis against upper respiratory tract infections for t least fiv years postoperatively combined with family planni g measures are recommended. This policy has apparently up till now sa ed five f the patients. The most interesting one is a case with an alveolar rhabdomyosarcoma, who seems to be the first reported in literature to live four years after the diagnosis was established.

Primary malignant tumors localized to the head and neck region in childhood are very rare Nevertheless, when they appear they always constitute great diagnostic, therapeutic and above all prognostic difficulties because such serious diseases in the early years of life involve a very oppressing family trauma. In this survey the morbidity frequency clinical course and prognosis of this type of tumor will be discussed. Eight cases with similar but yet clinically and pathologically different malignant tumors of the head and neck region in children below 15 years of age will be especially considered. The value of early properly planned surgical and radiological procedures combined with a carefully repeated follow-up is stressed. With this policy it may be possible to reduce the normally initial pessimistic out look.

MATERIAL

Malmö, with a well-defined population of about 250 000 inhabitants has only one general hospital and one department of pathology serving the entire area. The town, therefore offers exceptionally good possibilities for medico-demographic investigations. During the ten years 1958–1967 there were 39 deaths in the age group 0–14 years and the autopsy frequency in this group was as high as 82.8% or 612 cases. Malignant tumors were diagnosed in only 31 f the autopsied cases i.e 3 1% (Table 1)

It is clear that leukosis was the dominating cause of death (13 of the 31 cases i 41 9%) and there were only two cases (Table 2, cases 2 and 3) of

TABLE 1 *Malignant tumors during 10 years (1958–1967) among the 612 autopsied subjects below 15 years of age in a defined population with an autopsy frequency of 82 8 per cent*

	Leukoses		Glioma		Osteogenic sarcoma		Ewing's sarcoma		Sarcoma botryoides		Nephroblastoma		Rhabdomyosarcoma		Total	
Age group	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0–4	1	0	1	0	0	0	0	0	2	0	1	2	1	0	6	8
5–9	3	0	0	3	0	0	0	0	0	0	1	0	0	0	4	3
10–14	2	1	1	1	0	2	1	2	0	0	0	0	0	0	4	6
Total	6	7	2	4	0	2	1	2	2	0	2	2	1	0	14	17

special interest for the E.N T department. One of them had an embryonal rhabdomyosarcoma originating subcutaneously in the right mandibular angle involving the mandible and the floor of the mouth The second was a case of Ewing's sarcoma of the skull A third patient died of reticulum cell sarcoma of the neck but was unfortunately not autopsied (Table 2 case 1) In addition, another five cases with malignant tumors originating in the head and neck region were clinically diagnosed and treated at the hospital during the same period (Table 2, cases 4–8) They have all been exposed to more or less extended surgical intervention and three cases also received pre or postoperative radiotherapy All five are still alive after $1^{1}/_{2}$–6 years without symptoms of metastasis or recurrence

Case Reports

Case 7

B S Boy 6 years old without heredity for malignancy Repeated infections of the upper respiratory tract. In December 1965 the patient got a soft tissue tumor in the right upper part of the neck covering also the mastoid process. The tumor was not tender and looked like an atheroma A simple incision was therefore made and a partly necrotic mass obtained The histopathological examination however showed a fibrosarcoma. A radical en bloc-excision was made including the skin around the primary incision, the periost of the mastoid process, part of the sterno-cleido-mastoid muscle and the posterior part of the outer parotid lobe The operation was macroscopically radical and no radiotherapy was given

Microscopically An uncapsulated cellular tumor was found infiltrating the dermis, the subcutaneous fat and the superficial parts of the parotid gland. The cells had rather large nuclei some of them with prominent nucleoli, and a high mitotic frequency (Fig 1) The polymorphism was, however of moderate degree The cytoplasm was scanty and each cell was surrounded by reticulin fibres (Fig 2) In some parts small amounts of collagen were produced separating tumor cells usually growing in more or less regular bundles. There were no metastases in the regional lymph nodes.

TABLE 2 *Malignant head- and neck tumors in patients below 15 years of age in a defined population of 250 000 inhabitants during 10 years (1958–196[illegible])*

Case	Diagnosis	Age at discovery	Treatment: Surgery	Treatment: Radiotherapy	Prognosis
1. R. N.	Reticulum cell sarcoma of the neck (right)	7 months	—	X-ray 3700 R	Dead after 4 months
2. A. D.	Ewing's sarcoma of the skull	13 years	—	X-ray 4100 R (Sendoxan®)	Dead after 9 months
3. T. N.	Embryonal rhabdomyosarcoma of the gingiva (lower right)	3 years	—	X-ray 4000 R (– Sendoxan®)	Dead after 8 months
4. G. H.	Mucoepidermoid carcinoma of the left parotid gland	11 years	Radical parotidectomy including the deep portion	X-ray 4[illegible] R praeop – local radium application (90 mg/12 hours)	Alive after 9 years
5. T. K.	Malignant granulomatosis (Hodgkin) left of the neck	8 years	Radical neck dissection (left)	X-ray 2100 R (left) 2100 R (right) and 2100 R (left axilla) [illegible] 800 mg Sendoxan®	Alive after 8 years
6. P. O. J.	Bilateral lymphoreticulum cell sarcoma of the neck	6 years	Partial neck dissection bilaterally	X-ray 2000 R on bilateral fields	Alive after 1½ years
7. B. S.	Fibrosarcoma of the neck, mastoid and parotid region (right)	6 years	Radical surgery (See case report)	—	Alive after 3 years
8. P. S.	Alveolar rhabdomyosarcoma of the neck (right)	2 years	Radical surgery (See case report)	—	Alive after 4 years

Diagnosis Fibrosarcoma.

Prognosis The patient has been checked postoperatively every month and repeated prophylactical treatment with gammaglobulin (4 ml 12.5% human type KABI®) against infection has been given in spite of a normal serum electrophoresis. Antibiotics have also been prescribed prophylactically in periods of infections. The sedimentation rate has been normal. The differential white blood count has continuously showed an increased number of monocytes. Present observation time Three years without tumor spread or local recurrence.

Case 8

P. S. Boy two years old, with a family anamnesis of bronchial asthma and diabetes but no malignancy. From the first year of life he had several upper respiratory tract infections. In September 1964 during an infection

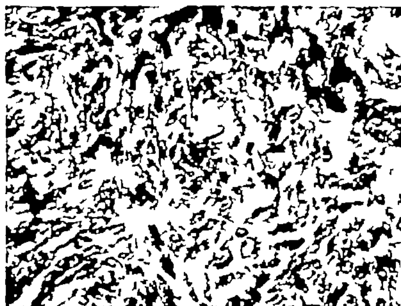


Fig 1 Case 7 Cellular fibrosarcoma with several mitoses (va Gieso 500)

he also got torticollis and swallowing difficulties. A tumor (3 × 4 cm) was palpated in the right upper part of the neck X ray pictures of the vertebral column cranium trachea and lungs were all normal The serum electrophoresis showed an active process with α_1 and α_2 rise and a positive ASTA (8.7 I.U.) was also present Repeated white cell counts showed a slight rise of monocytic cells (10%) A fine needle aspiration biopsy pointed to a malignant tumor At the following radical operation the periost of the



Fig 2 Case 7 Ret cull fibres surro ndi g practically all t mo cell (Gridl y 100)

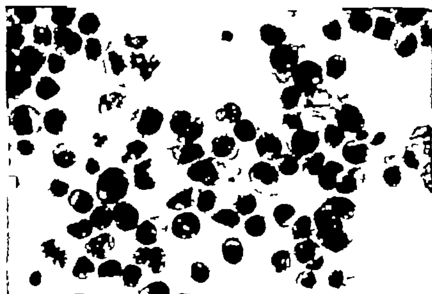


Fig 3 Case 8. Cell lar smea with m lti leated giant cells. One giant cell with pparent mitoses i ll nclei (Ma -Grünewald-Giemsa 480)

mastoid process, the upper part of the sternocleido-mastoid muscle, the internal jugular vein, the external carotid artery to the base of the skull, the submandibular gland and the surrounding soft tissues down to and in cluding the ends of the transverse processes of the second and third vertebrae were excised en bloc. The histological examination of the surgical specimen showed a sarcoma which, however was difficult to classify ini tially Therefore a therapeutically neglectable dose of telegamma was ini tially started (800 R) but not fulfilled when the diagnosis became established.

Fine needle aspiration biopsy showed mostly dissociated cells which had eccentric nuclei with irregular membranes and coarse chromatin. Some of the nuclei had one or more large nucleoli. The amount of cytoplasm varied and usually a perinuclear halo was found In many cells the cytoplasm was vacuolated Cross striations were not seen Giant cells with multiple nuclei or one bizarre, lobulated nucl us were easily recognized Mitoses were frequent (Figs. 3 and 4)

Preliminary diagnos s Unspecified malignant cells.

Histopathologically The tumor had an al eolar structure The alveoli were formed by fibrous septa of varying thickness In the more cellular parts of the tum the septa were often thin, built up of capillaries surrounded by a small am unt of fibrous tissue. In other parts th septa were thick and rich in reticul n fibres. The septa formed rounded or longated spaces lined with a single row of prominent tumor cell —"lining lls" The alveolar spaces

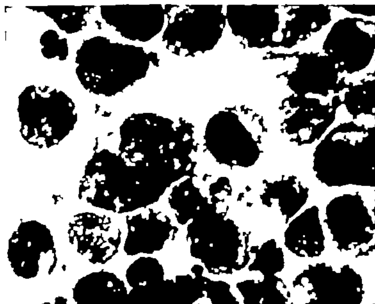


Fig 4 Case 8 Lobulated gia t cell nd plump cell with coarse chromatin nd vacuolated cytopl m (M v-Grunew ld-Gi msa 1200)

contained varying numbers of dissociated cells— floating cells" (Fig 5) Most of the tumor cells had a large eccentric, blunt or rounded nucleus with coarse chromatin Mitoses were frequent Giant cells of different types were seen. Some were multinucleated while others had a bizarre lobulated nucleus.

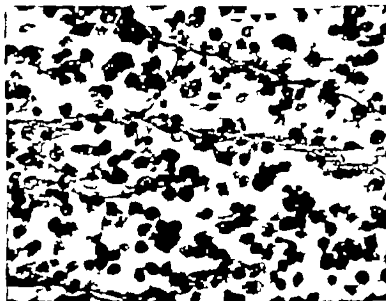


Fig 5 Case 8. Alveoli with "floating cells and protruding "lini g cell (H em t ylineosi 480)

The cytoplasm contained myofibril-like structures and was vacuolated in many of the cells.

Diagnosis Alveolar rhabdomyosarcoma.

Prognosis In spite of gammaglobulin every third week (4 cc 12.5% human type, KABI®) the boy had several upper respiratory tract infections. Antibiotics have been given about ten times. After $1^1/_2$ years there was a suspect recurrence Two rather big tumors appeared below the incision scar They were extirpated immediately and thoroughly examined histologically No recurrence of sarcoma could be identified, only suture granulomas. Present observation time Four years without any signs of spread or local recurrence of the tumor The boy develops well and has no movement inhibition of the neck.

DISCUSSION

With the exception of th neonatal period accidents constitute the most common cause of death in childhood, followed by malignant tumors and then by serious infections (Huth 1958) Among 730 autopsies in children aged 12 years or less Pack & Ariel (1954) report only 14 1% malignant tumors. In our autopsy material this figure was as low as 5 1% the half of which was due to leukosis. From a clinical material Cocchi (1958) concludes that only 2.9% of all malignant tumors appear in children below 15 years of age The dominance of sarcoma in this age group was striking This is also stated by Steiner (1954) who found only one case of carcinoma among 219 malignant tumors below 12 years of age.

In a series of 250 cases of malignancy in children Michael (1964) observed that lympho- and reticulum cell sarcoma with various localisation constituted 5.0% (13 cases) fibrosarcoma 3 1% (8 cases) and Ewing's sarcoma 1.9% (5 cases) Rhabdomyosarcomas were not mentioned at all in this paper The total number of tumors affecting the head and neck region was not given.

Malignant tumors appearing in the ENT-region in childhood are thus very rare and have therefore mostly been described as separate case reports in the literature (Schröder *et al* 1968) A broad statistical morbidity analysis is, however reported by Boehmühl & Herold (1968) These authors report only 27 malignant tumors in the ENT region of children among about 17 million inhabitants with totally 211 968 malignant tumors in East Germany during a five year period This means 0 03 cases per 100 000 inhabitants a year This figure is not in accordance with our material which is ten times higher i.e. 0.3 cases per 100 000 inhabitants a year In any case malignant tumors of the head and neck in childhood are extremely rare and the diagnosis may th refore easily be delayed. Markowicz & Shanon (1959) report an average duration of symptoms of 4.3 months in their material before th correct diagnosi was established. This delay naturally reduces the possibil ty of all therapeutic means. The difficulties are increased by the

fact that neglectable inflammatory lymph node swellings are so common among children Therefore in our opinion a preliminary diagnostic fine needle aspiration biopsy should be used more often in children who have more or less permanent lymph node enlargements or soft tissue swellings in t[illegible] head and neck region

Malignant lymphomas have obviously a predilection for originating in the [illegible]ck region Their prognosis is not good but instances have been reported, [illegible]g by Michael (1964) where local removal of a single lymph node or a [illegible]hain of lymph nodes has resulted in cure This is apparently also the case in our patients, numbers 4 and 6 Fibrosarcomas are histologically easily [illegible]nfused with fibromatoses and have a great local malignancy rate with a [illegible]nounced tendency for recurrence but a low metastasizing potential

Rhabd[illegible]myosarcomas, on the other hand are characterized by early and [illegible]te-spread metastases The outlook however seems not hopeless if early a[illegible]equ[illegible]te therapy is given except in most cases of the alveolar type of [illegible]odomy[illegible]arcomas (Mikulowski & Berge 1969) Only about 60 cases of [illegible] type [illegible]f [illegible]umor have hitherto been described in the literature and case 5 [illegible]n [illegible]ur [illegible]aterial seems to be the first to survive 4 years without signs of [illegible]rren[illegible] [illegible]r me[illegible] [illegible]ases This is probably the most important clinical ex[illegible]rience gained [illegible] [illegible]s investigation This case also supports the opinion of [illegible]aund & Pig[illegible] [illegible]t [illegible] [illegible]at patients with sarcomas of the head and neck [illegible]hould be tr[illegible] [illegible] [illegible]nded surgical excision whenever this is technically possibl[illegible] [illegible]ical procedures must however be determined as [illegible]arly as po[illegible] [illegible]nd see" policy is not to be recommended Operations in thi[illegible] a neck dissection—is easily tolerated by children but it is al[illegible] [illegible]o inform the parents that this operation might be necessary [illegible]nection with a simple lymph node extirpation, depending [illegible] [illegible] [illegible] [illegible]he frozen section diagnosis.

Radiothera[illegible] [illegible] [illegible]ndicated and should always be administered in cases with a [illegible] [illegible]itive tumor even if the surgeon reports a probably "radical [illegible] [illegible] [illegible]t radiotherapy can not replace surgery as stated by Kinsey *et al* (1[illegible] [illegible] [illegible]llows "In general it has been found that excisional therapy wh[illegible]n f[illegible]a[illegible]ble is superior to radiation therapy and that radiation therapy is the most useful for a non resectable or recurrent tumor and for tumors of the type which fall into the lymphoma class " This view is shared also by Pinkel & Pickren (1961) by Porterfield & Zimmerman (1962) and by Bardwill & McComb (1964)

Regular conferences including pathologist, radio-therapist, and surgeon are therefore necessary in clinics where these patients are being treated. It must also be remembered that all malignant tumors in childhood are individual and should be handled individually The three patients in whom radical surgery was judged impossible and who got only radiological treatment had a survival time of 4–9 months, whereas today the survival time of the cases who got radical surgery alone or combined with radiotherapy is $1^1/_2$–9 years.

The prognosis in the individual case is naturally impossible to predict. It

is due to (1) the type of tumor (2) the time delay of discovery (3) the efficiency and timing of surgery and radiotherapy and (4) the intensity of the follow up program.

Recurrences must be treated promptly Repeated upper respiratory tract infections may disguise or interfere with the tumor growth Prophylaxis against such infections may therefore be valuable and a monthly check up is easy to realize if it is fixed to such a prophylactic program (cases 7 and 8) which is recommended for at least 5 years. The prognosis following radi cal measures is not so bad as is sometimes stated but malignant tumors in early childhood always involve great family problems. If the patient belongs to a family with only one child, it may be wise to recommend another preg nancy at an early stage for all events. The burden of an unhappy outcome can then more easily be endured.

ZUSAMMENFASSUNG

Die Häufigkeit primärer Malignome des Kopfes und Halses im kindli hen Alt r wird an Hand einer wohldefinierten Population angegeben. Die Notwendigkeit der frühzeitigen Diagnose wird hervorgeh ben, wobei die Feinnadel-Aspiratio s-biopsie eine brauchbare Tech ik darstellt. Radikaloperation soll bei Möglichkeit ohn Verzug durchgeführt werden und in gegebenen Fällen u mitt lbar danach in däquaten Dosen radiologisch behandelt werden. Die Prognose scheint besser zu sein als im Allgem inen angenommen wird. Ein intensi es Nachuntersuchungs-programm sowie Prophylaxe gegen I fektionen der oberen Luftwege wird eben einer auf den Fall abgestimmten Familienplanierung die anschliessenden fü f Jahre nach der Operation mpfohlen Durch derartiges V rgehen k nnten llem Anschein nach fünf Patienten des Materials bisher gerettet werden. Der beachtens-werteste dieser Fälle ist ei alveoläres Rhabdomyosark m, welches laut Literatur angaben der erst Fall zu sein schei t, der seit sei er Entdeckung eine 4-J hres-periode überlebte.

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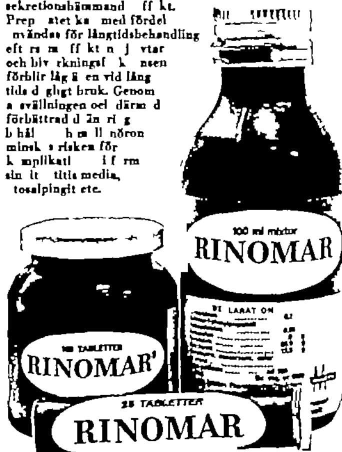
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